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For: INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS C VIRUS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

[X] This application claims priority from each of the following Application Nos./filing dates:
CIP U.S.S.N. 09/189,702, filed 11/10/98, which is a CIP of U.S.S.N. 08/205,713, filed 3/4/94, which is a CIP of 08/159,184, filed 11/29/93 and now abandoned, which is a CIP of 08/073,205, filed 6/4/93 and now abandoned, which is a CIP of 08/027,146, filed 3/5/93 and now abandoned
the disclosure(s) of which is (are) incorporated by reference.
Please amend this application by adding the following before the first sentence: "This application is a ☐ continuation ☐ continuation-in-part of and claims the benefit of U.S. Provisional Application No. 60/_____, filed _____, the disclosure of which is incorporated by reference."

Enclosed are:

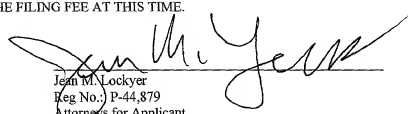
[X] 205 page(s) of specification
[X] 10 page(s) of claims
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☐ An assignment of the invention to _____
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In view of the Unsigned Declaration as filed with this application and pursuant to 37 CFR §1.53(f), Applicant requests deferral of the filing fee until submission of the Missing Parts of Application.

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PATENT APPLICATION

**INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS C VIRUS
USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS**

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Attorney Docket No.: 018623-014000US

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**INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS C VIRUS USING
PEPTIDE AND NUCLEIC ACID COMPOSITIONS****CROSS-REFERENCES TO RELATED APPLICATIONS**

This application is a Continuation-In-Part ("CIP") of U.S.S.N. 09/189,702 filed 11/10/98, which is a CIP of U.S.S.N. 08/205,713 filed 3/4/94, which is a CIP of 08/159,184 filed 11/29/93 and now abandoned, which is a CIP of 08/073,205 filed 6/4/93 and now abandoned, which is a CIP of 08/027,146 filed 3/5/93 and now abandoned. The present application is also related to U.S.S.N. 09/226,775, which is a CIP of U.S.S.N. 08/815,396, which claims the benefit of U.S.S.N. 60/013,113, now abandoned. Furthermore, the present application is related to U.S.S.N. 09/017,735, which is a CIP of abandoned U.S.S.N. 08/589,108; U.S.S.N. 08/753,622, U.S.S.N. 08/822,382, abandoned U.S.S.N. 60/013,980, U.S.S.N. 08/454,033, U.S.S.N. 09/116,424, and U.S.S.N. 08/349,177. The present application is also related to U.S.S.N. 09/017,524, U.S.S.N. 08/821,739, abandoned U.S.S.N. 60/013,833, U.S.S.N. 08/758,409, U.S.S.N. 08/589,107, U.S.S.N. 08/451,913, U.S.S.N. 08/186,266, U.S.S.N. 09/116,061, and U.S.S.N. 08/347,610, which is a CIP of U.S.S.N. 08/159,339, which is a CIP of abandoned U.S.S.N. 08/103,396, which is a CIP of abandoned U.S.S.N. 08/027,746, which is a CIP of abandoned U.S.S.N. 07/926,666. The present application is also related to U.S.S.N. 09/017,743, U.S.S.N. 08/753,615; U.S.S.N. 08/590,298, U.S.S.N. 09/115,400, and U.S.S.N. 08/452,843, which is a CIP of U.S.S.N. 08/344,824, which is a CIP of abandoned U.S.S.N. 08/278,634. The present application is also related to provisional U.S.S.N. 60/087,192 and U.S.S.N. 09/009,953, which is a CIP of abandoned U.S.S.N. 60/036,713 and abandoned U.S.S.N. 60/037,432. In addition, the present application is related to U.S.S.N. 09/098,584, U.S.S.N. 09/239,043, and to Provisional U.S. Patent Application 60/117,486 filed 1/27/99. The present application is also related to U.S. Patent Application entitled "Inducing Cellular Immune Responses to Hepatitis C Virus Using Peptide and Nucleic Acid Compositions", Attorney Docket No. 018623-0013910 filed 7/8/99. All of the above applications are incorporated herein by reference.

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This invention was funded, in part, by the United States government under grants with the National Institutes of Health. The U.S. government has certain rights in this invention.

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I. BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) infection is a global human health problem with approximately 150,000 new reported cases each year in the U.S. alone. HCV is a single stranded RNA virus, and is the etiological agent identified in most cases of non-A, non-B post-transfusion and post-transplant hepatitis, and is a common cause of acute sporadic hepatitis (Choo *et al.*, *Science* 244:359, 1989; Kuo *et al.*, *Science* 244:362, 1989; and Alter *et al.*, in: *Current Perspective in Hepatology*, p. 83, 1989). It is estimated that more than 50% of patients infected with HCV become chronically infected and, of those, 20% develop cirrhosis of the liver within 20 years (Davis *et al.*, *New Engl. J. Med.* 321:1501, 1989; Alter *et al.*, in: *Current Perspective in Hepatology*, p. 83, 1989; Alter *et al.*, *New Engl. J. Med.* 327:1899, 1992; and Dienstag, J. L. *Gastroenterology* 85:430, 1983). Moreover, the only therapy available for treatment of HCV infection is interferon- α . Most patients are unresponsive, however, and among the responders, there is a high recurrence rate within 6-12 months of cessation of treatment (Liang *et al.*, *J. Med. Virol.* 40:69, 1993). Ribavirin, a guanosine analog with a broad spectrum activity against many RNA and DNA viruses, has been shown in clinical trials to be effective against chronic HCV infection when used in combination with interferon- α (see, e.g., Poynard *et al.*, *Lancet* 352:1426-1432, 1998; Reichard *et al.*, *Lancet* 351:83-87, 1998). However, the response rate is still well below 50%.

Virus-specific, human leukocyte antigen (HLA) class I-restricted cytotoxic T lymphocytes (CTL) are known to play a major role in the prevention and clearance of virus infections *in vivo* (Oldstone *et al.*, *Nature* 321:239, 1989; Jamieson *et al.*, *J. Virol.* 61:3930, 1987; Yap *et al.*, *Nature* 273:238, 1978; Lukacher *et al.*, *J. Exp. Med.* 160:814, 1994; McMichael *et al.*, *N. Engl. J. Med.* 309:13, 1983; Sethi *et al.*, *J. Gen. Virol.* 64:443, 1983; Watari *et al.*, *J. Exp. Med.* 165:459, 1987; Yasukawa *et al.*, *J. Immunol.* 143:2051, 1989; Tigges *et al.*, *J. Virol.* 66:1622, 1993; Reddenhase *et al.*, *J. Virol.* 55:263, 1985; Quinnan *et al.*, *N. Engl. J. Med.* 307:6, 1982). HLA class I molecules are expressed on the surface of almost all nucleated cells. Following intracellular processing of antigens, epitopes from the antigens are presented as a complex with the HLA class I molecules on the surface of such cells. CTL recognize the peptide-HLA class I complex, which then results in the destruction of the cell bearing the HLA-peptide complex directly by the CTL and/or via the activation of non-destructive mechanisms e.g., the production of interferon, that inhibit viral replication.

In view of the heterogeneous immune response observed with HCV infection, induction of a multi-specific cellular immune response directed simultaneously against multiple HCV epitopes appears to be important for the development of an efficacious vaccine against HCV. There is a need, however, to establish vaccine embodiments that elicit immune responses that correspond to responses seen in patients that clear HCV infection.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

This invention applies our knowledge of the mechanisms by which antigen is recognized by T cells, for example, to develop epitope-based vaccines directed towards HCV. More specifically, this application communicates our discovery of specific epitope pharmaceutical compositions and methods of use in the prevention and treatment of HCV infection.

Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. There is evidence that the immune response to whole antigens is directed largely toward variable regions of the antigen, allowing for immune escape due to mutations. The epitopes for inclusion in an epitope-based vaccine are selected from conserved regions of viral or tumor-associated antigens, which thereby reduces the likelihood of escape mutants. Furthermore, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines.

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen. Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from that pathogen in a vaccine composition. A

5 “pathogen” may be an infectious agent or a tumor associated molecule.

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used

10 that are specific for HLA molecules corresponding to each individual HLA allele, therefore, impractically large numbers of epitopes would have to be used in order to cover ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The greater the number of HLA antigen molecules bound, the greater the breadth of

15 population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, for example, so that peptides that are able to bind to multiple HLA antigens do so with an affinity that will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that

20 correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

In a preferred embodiment, epitopes for inclusion in vaccine compositions of the

25 invention are selected by a process whereby protein sequences of known antigens are evaluated for the presence of motif or supermotif-bearing epitopes. Peptides corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those peptides that bind at an intermediate or high affinity *i.e.*, an IC_{50} (or a K_D value) of 500

30 nM or less for HLA class I molecules or 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response. Immunogenic peptide epitopes are selected for inclusion in vaccine compositions.

Supermotif-bearing peptides may additionally be tested for the ability to bind to multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be

analogued to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes an embodiment comprising a method for monitoring or evaluating an immune response to HCV in a patient having a known HLA-type, the method comprising incubating a T lymphocyte sample from the patient with a peptide composition comprising an HCV epitope consisting essentially of an amino acid sequence described in Tables VII to Table XX or Table XXII which binds the product of at least one HLA allele present in said patient, and detecting for the presence of a T lymphocyte that binds to the peptide. A CTL peptide epitope may, for example, comprise a tetrameric complex.

An alternative modality for defining the peptide epitopes in accordance with the invention is to recite the physical properties, such as length; primary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptide epitopes is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (e.g. pocket configuration and charge distribution) and reciting that the peptide epitope fits and binds to said pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Figure 1 provides a graph of total frequency of genotypes as a function of the number of HCV candidate epitopes bound by HLA-A and B molecules, in an average population.

Figure 2: Figure 2 illustrates the position of peptide epitopes in an experimental model minigene construct.

IV. DETAILED DESCRIPTION OF THE INVENTION

The peptide epitopes and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to HCV by stimulating the production of CTL or HTL responses. The peptide epitopes, which are derived directly or indirectly from native HCV amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to HCV. The complete polypeptide sequence from HCV

and its variants can be obtained from Genbank. Peptide epitopes and analogs thereof can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of HCV, as will be clear from the disclosure provided below.

- 5 The peptide epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is that analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immunogenicity. Further, the present invention provides compositions and combinations of compositions
- 10 that enable epitope-based vaccines that are capable of interacting with HLA molecules encoded by various genetic alleles to provide broader population coverage than prior vaccines.

IV.A. Definitions

- 15 The invention can be better understood with reference to the following definitions, which are listed alphabetically:

- A "computer" or "computer system" generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a
- 20 touch screen, or a microphone; and display structure. Additionally, the computer may include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

- 25 A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

- A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, e.g., Sercarz, *et al.*, *Annu. Rev. Immunol.*
- 30 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins

and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably. It is to be appreciated, however, that isolated or purified protein or peptide molecules larger than and comprising an epitope of the invention are still within the bounds of the invention.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see, e.g., Stites, et al., IMMUNOLOGY*, 8TH ED., Lange Publishing, Los Altos, CA (1994).

An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like supertype molecules (where xx denotes a particular HLA type), are synonyms.

Throughout this disclosure, results are expressed in terms of "IC₅₀'s." IC₅₀ is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.,* limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. It should be noted that IC₅₀ values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.,* HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC₅₀ of a given ligand.

Assays for determining binding are described in detail, *e.g.,* in PCT publications WO 94/20127 and WO 94/03205. Alternatively, binding is expressed relative to a reference peptide. As a particular assay becomes more, or less, sensitive, the IC₅₀'s of the peptides tested may change somewhat. However, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC₅₀ of the reference peptide increases 10-fold, the IC₅₀ values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC₅₀, relative to the IC₅₀ of a standard peptide.

- Binding may also be determined using other assay systems including those using live cells (e.g., Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 1990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (e.g.,
- 5 Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (e.g., Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA systems (e.g., Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (e.g., Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or
- 10 assembly (e.g., Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

- As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC_{50} , or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 50 and about 500 nM. "High affinity" with
- 15 respect to binding to HLA class II molecules is defined as binding with an IC_{50} or K_D value of 100 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 100 and about 1000 nM.

- The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a
- 20 specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

- An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and
- 25 induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response, or a helper T cell response, to the antigen from which the immunogenic peptide is derived.

- The phrases "isolated" or "biologically pure" refer to material which is
- 30 substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

“Major Histocompatibility Complex” or “MHC” is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, *FUNDAMENTAL IMMUNOLOGY*, 3RD ED., Raven Press, New York, 1993.

The term “motif” refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A “negative binding residue” is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide’s corresponding HLA molecule.

The term “peptide” is used interchangeably with “oligopeptide” in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing peptides of the invention are 13 residues or less in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

“Pharmaceutically acceptable” refers to a non-toxic, inert, and physiologically compatible composition.

A “primary anchor residue” is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a “motif” for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, the primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 9-residue peptide epitope in accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table 1. For example, analog peptides can

be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

“Promiscuous recognition” is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding is synonymous with cross-reactive binding.

A “protective immune response” or “therapeutic immune response” refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term “residue” refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

A “secondary anchor residue” is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at “secondary anchor positions.” A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A “subdominant epitope” is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

A “supermotif” is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA antigens.

“Synthetic peptide” refers to a peptide that is not naturally occurring, but is man-made using such methods as chemical synthesis or recombinant DNA technology.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G.

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15 Symbols for the amino acids are shown below.

Single Letter Symbol	Three Letter Symbol	Amino Acids
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

IV.B. Stimulation of CTL and HTL responses

- The mechanism by which T cells recognize antigens has been delineated during the past ten years. Based on our understanding of the immune system we have developed efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to HCV in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of immunology-related technology is provided.
- A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601,

- 1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described herein and are set forth in Tables I, II, and III (*see also, e.g., Southwood, et al., J. Immunol.* 160:3363, 1998; Rammensee, *et al., Immunogenetics* 41:178, 1995; Rammensee *et al., SYFPEITHI*, access via web at : <http://134.2.96.221/scripts.hlaserver.dll/home.htm>; Sette, A. and Sidney, *J. Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, *J. Curr. Biol.* 6:52, 1994; Ruppert *et al., Cell* 74:929-937, 1993; Kondo *et al., J. Immunol.* 155:4307-4312, 1995; Sidney *et al., J. Immunol.* 157:3480-3490, 1996; Sidney *et al., Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, *J. Immunogenetics*, in press, 1999).

- Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See, e.g., Madden, D.R. Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al., Immunity* 4:203, 1996; Fremont *et al., Immunity* 8:305, 1998; Stern *et al., Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al., Nature* 364:33, 1993; Guo, H. C. *et al., Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al., Nature* 360:364, 1992; Silver, M. L. *et al., Nature* 360:367, 1992; Matsumura, M. *et al., Science* 257:927, 1992; Madden *et al., Cell* 70:1035, 1992; Fremont, D. H. *et al., Science* 257:919, 1992; Saper, M. A., Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

- Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA antigen(s).

- The present inventors have found that the correlation of binding affinity with immunogenicity, which is disclosed herein, is an important factor to be considered when evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, antigenicity, and immunogenicity.

Various strategies can be utilized to evaluate immunogenicity, including:

- 1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.,* Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.*, a ^{51}Cr -release assay involving peptide sensitized target cells.
- 2) Immunization of HLA transgenic mice (*see, e.g.,* Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, *e.g.*, a ^{51}Cr -release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.
- 3) Demonstration of recall T cell responses from immune individuals who have effectively been vaccinated, recovered from infection, and/or from chronically infected patients (*see, e.g.,* Rehmann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997). In applying this strategy, recall responses are detected by culturing PBL from subjects that have been naturally exposed to the antigen, for instance through infection, and thus have generated an immune response "naturally", or from patients who were vaccinated against the infection. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

The following describes the peptide epitopes and corresponding nucleic acids of the invention.

IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

As indicated herein, the large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of

5 binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or

10 better (*i.e.*, the value is ≤ 500 nM). HTL-inducing peptides preferably include those that have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is $\leq 1,000$ nM). For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected

15 peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in vaccines or in cellular screening analyses.

As disclosed herein, higher HLA binding affinity is correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways.

20 Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. In accordance with these principles, close to 90% of high binding peptides have been found to be

25 immunogenic, as contrasted with about 50% of the peptides which bind with intermediate affinity. Moreover, higher binding affinity peptides leads to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high affinity binding peptide is used. Thus, in preferred embodiments of the invention, high affinity binding epitopes are particularly useful.

30 The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (*see, e.g.*,

- Sette, *et al.*, *J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL from acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity threshold value of approximately 500 nM (preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses (*see, e.g., Schaeffer et al. Proc. Natl. Acad. Sci. USA* 86:4649-4653, 1989).

- An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g., Southwood et al. J. Immunology* 160:3363-3373, 1998, and U.S.S.N. 60/087192 filed 5/29/98). In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e.*, the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinity values of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinity values in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC_{50} of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

- The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

- In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets.

For HLA molecule pocket analyses, the residues comprising the B and F pockets of HLA class I molecules as described in crystallographic studies were analyzed (*see,*

- e.g., Guo, H. C. *et al.*, *Nature* 360:364, 1992; Saper, M. A., Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991; Madden, D. R., Garboczi, D. N. and Wiley, D. C., *Cell* 75:693, 1993; Parham, P., Adams, E. J., and Arnett, K. L., *Immunol. Rev.* 143:141, 1995). In these analyses, residues 9, 45, 63, 66, 67, 70, and 99 were considered to make up the B pocket; and the B pocket was deemed to determine the specificity for the amino acid residue in the second position of peptide ligands. Similarly, residues 77, 80, 81, and 116 were considered to determine the specificity of the F pocket; the F pocket was deemed to determine the specificity for the C-terminal residue of a peptide ligand bound by the HLA class I molecule.
- Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs. From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecule with high or intermediate affinity. Of these 22 peptides, 20 (*i.e.* 91%) were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques eliminates screening of 90% of the potential epitopes in a target antigen protein sequence.
- Such peptide epitopes are identified in the Tables described below.
- Peptides of the present invention may also comprise epitopes that bind to MHC class II DR molecules. A greater degree of heterogeneity in both size and binding frame position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands. This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I

counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the major energy of binding is contributed by peptide residues complexed with complementary pockets on the DRB*0101 molecules. An important anchor residue engages the deepest hydrophobic pocket (*see, e.g.,* Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) and is referred to as position 1 (P1). P1 may represent the N-terminal residue of a class II binding peptide epitope, but more typically is flanked towards the N-terminus by one or more residues. Other studies have also pointed to an important role for the peptide residue in the 6th position towards the C-terminus, relative to P1, for binding to various DR molecules.

Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs (*see, e.g.,* Tables I-III). If the presence of the motif corresponds to the ability to bind several allele-specific HLA antigens, it is referred to as a supermotif. The HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype."

The peptide motifs and supermotifs described below, and summarized in Tables I-III, provide guidance for the identification and use of peptide epitopes in accordance with the invention.

Examples of peptide epitopes bearing a respective supermotif or motif are included in Tables as designated in the description of each motif or supermotif below.

The Tables include a binding affinity ratio listing for some of the peptide epitopes. The ratio may be converted to IC₅₀ by using the following formula: IC₅₀ of the standard peptide/ratio = IC₅₀ of the test peptide (*i.e.,* the peptide epitope). The IC₅₀ values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC₅₀ values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding assays described herein are examples of standards; alternative standard peptides can also be used when performing such an analysis.

To obtain the peptide epitope sequences listed in each Table, protein sequence data from fourteen HCV isolates were evaluated for the presence of the designated supermotif or motif. The fourteen strains include HPCCGAA, HPCPLYPRE, HCV-H-CMR, HCV-J1, HPCGENANTI, HPCGENOM, HPCCHUMR, HPCJCG, HPCJTA, HCV-J483, HCV-JK1, HCV-N, HPCPOLP, and HCV-J8. Peptide epitopes were additionally evaluated on the basis of their conservancy among these fourteen strains. A criterion for conservancy requires that the entire sequence of an HLA class I binding peptide be totally

conserved in 79% of the sequences available for a specific protein. Similarly, a criterion for conservancy requires that the entire 9-mer core region of an HLA class II binding peptide be totally conserved in 79% of the sequences available for a specific protein. The percent conservancy of the selected peptide epitopes is indicated on the Tables. The frequency, *i.e.* the number of strains of the fourteen strains in which the totally conserved peptide sequence was identified, is also shown. The "position" column in the Tables designates the amino acid position of the HCV polyprotein that corresponds to the first amino acid residue of the epitope. The "number of amino acids" indicates the number of residues in the epitope sequence.

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI.

IV.D.1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, or M) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) is comprised of at least A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A1 supermotif are set forth on the attached Table VII.

IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA-A2.1 molecules (Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992) and cross-reactive binding within the HLA A2 family (Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.* 39:155-162, 1994) have been described. The present inventors have defined additional primary anchor residues that determine cross-reactive binding to multiple allele-specific HLA A2 molecules (Ruppert *et al.*, *Cell* 74:929-937, 1993; Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). The HLA-A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as a primary anchor residue at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise an A2 supermotif are set forth on the attached Table VIII. The motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.3. HLA-A3 supermotif

The HLA-A3 supermotif is characterized by the presence in peptide ligands of A, L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope (*e.g.*, in position 9 of 9-mers). Exemplary members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA molecules predicted to be members of the A3 superfamily are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids

at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A3 supermotif are set forth on the attached Table IX.

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IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue as a primary anchor in position 2, and Y, F, W, L, I, or M as primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e.*, the A24 supertype) includes at least A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

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Representative peptide epitopes that comprise the A24 supermotif are set forth on the attached Table X.

IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind the B7 supermotif (*i.e.*, the HLA-B7 supertype) is comprised of at least twenty six HLA-B proteins including: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (*see, e.g.*, Sidney, *et al.*, *J. Immunol.* 154:247, 1995; Barber, *et al.*, *Curr. Biol.* 5:179, 1995; Hill, *et al.*, *Nature* 360:434, 1992; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995). Other allele-specific HLA molecules predicted to be members of the B7 superfamily are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

30

Representative peptide epitopes that comprise the B7 supermotif are set forth on the attached Table XI.

IV.D.6. HLA-B27 supermotif

- 5 The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least
- 10 B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the
- 15 supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth on the attached Table XII.

IV.D.7. HLA-B44 supermotif

- 20 The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.*, the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4006. Peptide binding to each of the
- 25 allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the supermotif.

30 IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope. Exemplary members of the corresponding

family of HLA molecules that bind to the B58 supermotif (*i.e.*, the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific HLA molecules predicted to be members of the B58 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B58 supermotif are set forth on the attached Table XIII.

10 **IV.D.9. HLA-B62 supermotif**

The HLA-B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, I, or P) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the epitope. Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least: B*1501, B*1502, B*1513, and B5201. Other allele-specific HLA molecules predicted to be members of the B62 superfamily are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B62 supermotif are set forth on the attached Table XIV.

IV.D.10. HLA-A1 motif

The HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif is characterized by a primary anchor residue at position 3 rather than position 2. This motif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise either A1 motif are set forth on the attached Table XV. Those epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII.

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IV.D.11. HLA-A*0201 motif

- An HLA-A2*0201 motif was first determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9-residue peptide (Falk *et al.*,
 10 *Nature* 351:290-296, 1991). The A*0201 motif was also determined to further comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (Hunt *et al.*, *Science* 255:1261-1263, March 6, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992). Subsequently, the A*0201 allele-specific motif has been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2,
 15 and M as a primary anchor residue at the C-terminal position of the epitope. Additionally, the A*0201 allele-specific motif has been found to comprise a T at the C-terminal position (Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-
 20 terminal position of the epitope. The preferred and tolerated residues that characterize the primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, *see, e.g.*, Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Ruppert *et al.*, *Cell* 74:929-937, 1993; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-
 25 482, 1998). Secondary anchor residues that characterize the A*0201 motif have additionally been defined as disclosed herein. These are disclosed in Table II. Peptide binding to HLA-A*0201 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.
 30 Representative peptide epitopes that comprise an A*0201 motif are set forth on the attached Table VIII. The A*0201 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12. HLA-A3 motif

The HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, Y, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope.

- 5 Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A3 motif are set forth on the attached Table XVI. Those peptide epitopes that also comprise the A3 supermotif are
10 also listed in Table IX.

IV.D.13. HLA-A11 motif

The HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or
15 H as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A11 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A11 motif are set forth on the attached Table XVII; peptide epitopes comprising the A3 allele-specific motif are also
20 present in this Table because of the extensive overlap between the A3 and A11 motif primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

25 The HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope. Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the motif.

30 Representative peptide epitopes that comprise the A24 motif are set forth on the attached Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-A24-supermotif-bearing peptide epitopes.

Motifs Indicative of Class II HTL Inducing Peptide Epitopes

The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

5 IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701. Collectively, the common residues from these motifs delineate the HLA DR-1-4-7 supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V, I, L, or M) as a primary anchor residue in position 6 of a 9-mer core region. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified. These are set forth in Table III. Peptide binding to HLA- DRB1*0401, DRB1*0101, and/or DRB1*0701 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

- Conserved peptide epitopes *i.e.*, conserved in $\geq 79\%$ ($\geq 11/14$) of the HCV strains used for the present analysis, may be described as corresponding to epitopes containing a nine residue core comprising the DR-1-4-7 supermotif, and in which the 9 residue core is conserved in $\geq 79\%$ (wherein position 1 of the motif is at position 1 of the nine residue core). Conserved 9-mer core regions are set forth in Table XIXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a conserved nine residue core, are also shown in section "a" of the Table. Cross-reactive binding data for exemplary 15-residue supermotif-bearing peptides are shown in Table XIXb.

IV.D.16. HLA DR3 motifs

- Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules. In the first motif (submotif DR3A) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1 of a 9-mer core, and D is present as an anchor at position 4, towards the carboxyl terminus of the epitope. As in other class II motifs, core position 1 may or may not occupy the peptide N-terminal position.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif

5 DR3B): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Conserved 9-mer core regions (*i.e.*, those sequences that are conserved in at least

10 79% of the 14 HCV strains used for the analysis) corresponding to a nine residue sequence comprising the DR3A submotif (wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a conserved nine residue core, are also shown in Table XXa. Table XXb shows binding data of exemplary

15 DR3 submotif A-bearing peptides.

Conserved 9-mer core regions (*i.e.*, those that are at least 79% conserved in the 14 HCV strains used for the analysis) comprising the DR3B submotif and respective exemplary 15-mer peptides comprising the DR3 submotif-B epitope are set forth in Table XXc. Table XXd shows binding data of exemplary DR3 submotif B-bearing peptides.

Each of the HLA class I or class II peptide epitopes set out in the Tables herein are deemed singly to be an inventive aspect of this application. Further, it is also an inventive aspect of this application that each peptide epitope may be used in combination with any other peptide epitope.

25 IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the peptides of the invention (and nucleic acid compositions that encode such peptides) through selecting peptide epitopes that bind to

30 HLA alleles which, when considered in total, are present in most of the population. Table XXI lists the overall frequencies of the HLA class I supertypes in various ethnicities (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7-supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on the average of over 40% in each of these five major ethnic groups. Coverage in excess of

80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are present, on average, in a range from 25% to 40% in these major ethnic populations (Table XXIa). While less prevalent overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one major ethnic group (Table XXIa). Table XXIb summarizes the estimated prevalence of combinations of HLA supertypes that have been identified in five major ethnic groups. The incremental coverage obtained by the inclusion of A1-, A24-, and B44-supertypes to the A2, A3, and B7 coverage, or all of the supertypes described herein, is shown.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups..

IV.F. Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few "immunodominant" determinants (Zinkernagel, *et al.*, *Adv. Immunol.* 27:5159, 1979; Bennink, *et al.*, *J. Exp. Med.* 168:1935-1939, 1988; Rawle, *et al.*, *J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al.*, *Science* 175:273-279, 1972) could be explained by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello, *et al.*, *J. Immunol.* 131:1635, 1983); Rosenthal, *et al.*, *Nature* 267:156-158, 1977), or to be selectively recognized by the existing TCR (T cell receptor) specificities (repertoire theory) (Klein, J., *IMMUNOLOGY, THE SCIENCE OF SELF/NONSELF DISCRIMINATION*, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993).

The concept of dominance and subdominance is relevant to immunotherapy of both infectious diseases and cancer. For example, in the course of chronic viral disease, recruitment of subdominant epitopes can be important for successful clearance of the infection, especially if dominant CTL or HTL specificities have been inactivated by functional tolerance, suppression, mutation of viruses and other mechanisms (Franco, *et al.*, *Curr. Opin. Immunol.* 7:524-531, 1995). In the case of cancer and tumor antigens, CTLs recognizing at least some of the highest binding affinity peptides might be functionally inactivated. Lower binding affinity peptides are preferentially recognized at these times, and may therefore be preferred in therapeutic or prophylactic anti-cancer vaccines.

In particular, it has been noted that a significant number of epitopes derived from known non-viral tumor associated antigens (TAA) bind HLA class I with intermediate affinity (IC_{50} in the 50-500 nM range). For example, it has been found that 8 of 15 known TAA peptides recognized by tumor infiltrating lymphocytes (TIL) or CTL bound in the 50-500 nM range. (These data are in contrast with estimates that 90% of known viral antigens were bound by HLA class I molecules with IC_{50} of 50 nM or less, while only approximately 10% bound in the 50-500 nM range (Sette, *et al.*, *J. Immunol.*, 153:558-5592, 1994). In the cancer setting this phenomenon is probably due to elimination or functional inhibition of the CTL recognizing several of the highest binding peptides, presumably because of T cell tolerization events.

Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow existing T cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response. This ability would greatly enhance the usefulness of peptide-based vaccines and therapeutic agents.

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other properties of the peptides such as binding affinity or peptide stability. Having established

the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in co-pending U.S.S.N. 09/226,775 filed 1/6/99.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, and in many cases secondary anchors. Analog peptides can be created by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Tables II and III). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of analyzed peptides, the incidence of cross-reactivity increases from 22% to 37% (*see, e.g.*, Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues associated with high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the

- immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the case of class II epitopes only, cells that have been pulsed with whole protein antigens, to establish
- 5 whether endogenously produced antigen is also recognized by the relevant T cells.

- Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I binding peptides exhibiting binding affinities of 500-5000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by
- 10 substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

- Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in, e.g., a liquid environment. This substitution may occur at any position of the peptide
- 15 epitope. For example, a cysteine (C) can be substituted out in favor of α -amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α -amino butyric acid for C not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (see, e.g., the review by Sette *et al.*, In:
- 20 Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999). Substitution of cysteine with α -amino butyric acid may occur at any residue of a peptide epitope, i.e. at either anchor or non-anchor positions.

- Representative analog peptides are set forth in Table XXII. The Table indicates the length and sequence of the analog peptide as well as the motif or supermotif, if
- 25 appropriate. The information in the "Fixed Nomenclature" column indicates the residues substituted at the indicated position numbers for the respective analog.

IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Bearing Peptides

- 30 In order to identify supermotif- or motif-bearing epitopes in a target antigen, a native protein sequence, e.g., a tumor-associated antigen, or sequences from an infectious organism, or a donor tissue for transplantation, is screened using a means for computing, such as an intellectual calculation or a computer, to determine the presence of a supermotif or motif within the sequence. The information obtained from the analysis of

native peptide can be used directly to evaluate the status of the native peptide or may be utilized subsequently to generate the peptide epitope.

Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. For example, the target molecules considered herein include, without limitation, the core, S, E1, NS1/E2, NS2, NS3, NS4, and NS5 regions of HCV.

In cases where the sequence of multiple variants of the same target protein are available, peptides may also be selected on the basis of their conservancy. A presently preferred criterion for conservancy defines that the entire sequence of an HLA class I binding peptide or the entire 9-mer core of a class II binding peptide, be totally (*i.e.*, 100%) conserved in at least 79% of the sequences evaluated for a specific protein. This definition of conservancy has been employed herein; although, as appreciated by those in the art, lower or higher degrees of conservancy can be employed as appropriate for a given antigenic target.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (see, *e.g.*, Ruppert, J. *et al. Cell* 74:929, 1993). However, by extensively analyzing peptide-HLA binding data disclosed herein, data in related patent applications, and data in the art, the present inventors have developed a number of allele-specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2j} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described, for example, in Gulukota, K. *et al.*, *J. Mol. Biol.* 267:1258, 1997.

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs (see, e.g., Milik *et al.*, *Nature Biotechnology* 16:753, 1998; Altuvia *et al.*, *Hum. Immunol.* 58:1, 1997; Altuvia *et al.*, *J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al.*, *Bioinformatics* 14:121-130, 1998; Parker *et al.*, *J. Immunol.* 152:163, 1993; Meister *et al.*, *Vaccine* 13:581, 1995; Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994; Sturniolo *et al.*, *Nature Biotechnol.* 17:555 1999).

For example, it has been shown that in sets of A*0201 motif-bearing peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, 69% of the peptides will bind A*0201 with an IC_{50} less than 500 nM (Ruppert, J. *et al. Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS" program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. The identified peptides can be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles. As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (e.g., without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences.

In accordance with the procedures described above, HCV peptide epitopes and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules have been identified (Tables VII-XX; Table XXII).

5 **IV.H. Preparation of Peptide Epitopes**

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of
 10 other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side
 15 chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

Desirably, the peptide epitope will be as small as possible while still maintaining substantially all of the immunologic activity of the native protein. When possible, it may
 20 be desirable to optimize HLA class I binding peptide epitopes of the invention to a length of about 8 to about 13 amino acid residues, preferably 9 to 10. HLA class II binding peptide epitopes may be optimized to a length of about 6 to about 30 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptide epitopes are commensurate in size with endogenously processed pathogen-derived
 25 peptides or tumor cell peptides that are bound to the relevant HLA molecules.

The identification and preparation of peptides of other lengths can also be carried out using the techniques described herein. Moreover, it is preferred to identify native peptide regions that contain a high concentration of class I and/or class II epitopes. Such a sequence is generally selected on the basis that it contains the greatest number of
 30 epitopes per amino acid length. It is to be appreciated that epitopes can be present in a frame-shifted manner, *e.g.* a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; upon intracellular processing, each epitope can be exposed and bound by an HLA molecule upon administration of such a

peptide. This larger, preferably multi-epitopic, peptide can be generated synthetically, recombinantly, or via cleavage from the native source.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (See, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984). Further, individual peptide epitopes can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

The nucleotide coding sequence for peptide epitopes of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al.*, *J. Am. Chem. Soc.* 103:3185 (1981). Peptide analogs can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence; exemplary nucleic acid substitutions are those that encode an amino acid defined by the motifs/super motifs herein. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

IV.I. Assays to Detect T-Cell Responses

- Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are synthesized and tested for their ability to bind to the appropriate HLA proteins. These assays may involve evaluating the binding of a peptide of the invention to purified HLA class I molecules in relation to the binding of a radioiodinated reference peptide. Alternatively, cells expressing empty class I molecules (*i.e.* lacking peptide therein) may be evaluated for peptide binding by immunofluorescent staining and flow microfluorimetry. Other assays that may be used to evaluate peptide binding include peptide-dependent class I assembly assays and/or the inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule, typically with an affinity of 500 nM or less, are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with selected target cells associated with a disease. Corresponding assays are used for evaluation of HLA class II binding peptides. HLA class II motif-bearing peptides that are shown to bind, typically at an affinity of 1000 nM or less, are further evaluated for the ability to stimulate HTL responses.

- Conventional assays utilized to detect T cell responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL

activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence was derived.

- 5 More recently, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining for intracellular lymphokines, and interferon release assays or ELISPOT assays.
- 10 Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp. Med.* 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

- 15 HTL activation may also be assessed using such techniques known to those in the art such as T cell proliferation and secretion of lymphokines, *e.g.* IL-2 (*see, e.g.* Alexander *et al.*, *Immunity* 1:751-761, 1994).

- 20 Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11 (which can additionally be used to analyze HLA-A3 epitopes), and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. Mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-
- 25 pulsed target cells and target cells transfected with appropriate genes. CTL responses may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

Exemplary immunogenic peptide epitopes are set out in Table XXIII.

30 **IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune Responses**

HLA class I and class II binding peptides as described herein can be used, in one embodiment of the invention, as reagents to evaluate an immune response. The immune response to be evaluated may be induced by using as an immunogen any agent that may

result in the production of antigen-specific CTLs or HTLs that recognize and bind to the peptide epitope(s) to be employed as the reagent. The peptide reagent need not be used as the immunogen. Assay systems that may be used for such an analysis include relatively recent technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays.

For example, a peptide of the invention may be used in a tetramer staining assay to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a pathogen or immunogen. The HLA-tetrameric complex is used to directly visualize antigen-specific CTLs (see, e.g., Ogg *et al.*, *Science* 279:2103-2106, 1998; and Altman *et al.*, *Science* 174:94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood mononuclear cells. A tetramer reagent using a peptide of the invention may be generated as follows: A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and β_2 -microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells may then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes.

Peptides of the invention may also be used as reagents to evaluate immune recall responses. (see, e.g., Berton *et al.*, *J. Clin. Invest.* 100:503-513, 1997 and Penna *et al.*, *J. Exp. Med.* 174:1565-1570, 1991.) For example, patient PBMC samples from individuals with HCV infection may be analyzed for the presence of antigen-specific CTLs or HTLs using specific peptides. A blood sample containing mononuclear cells may be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population may be analyzed, for example, for cytotoxic activity (CTL) or for HTL activity.

The peptides may also be used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen may be analyzed using, for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele-specific molecules present in that patient are selected for the analysis. The immunogenicity of the vaccine is indicated by the presence of HCV epitope-specific CTLs and/or HTLs in the PBMC sample.

- The peptides of the invention may also be used to make antibodies, using techniques well known in the art (see, e.g. *CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Manual* Harlow, Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989), which may be useful as reagents to
- 5 diagnose HCV infection. Such antibodies include those that recognize a peptide in the context of an HLA molecule, i.e., antibodies that bind to a peptide-MHC complex.

IV.K. Vaccine Compositions

- Vaccines that contain an immunogenically effective amount of one or more
- 10 peptides as described herein are a further embodiment of the invention. Once appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (e.g., Vitiello, A. et al., *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-
- 15 glycolide) ("PLG") microspheres (see, e.g., Eldridge, et al., *Molec. Immunol.* 28:287-294, 1991; Alonso et al., *Vaccine* 12:299-306, 1994; Jones et al., *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (see, e.g., Takahashi et al., *Nature* 344:873-875, 1990; Hu et al., *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (see e.g., Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), viral
- 20 delivery vectors (Perkus, M. E. et al., In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. et al., *Nature* 320:535, 1986; Hu, S. L. et al., *Nature* 320:537, 1986; Kieny, M.-P. et al., *AIDS Bio/Technology* 4:790, 1986; Top, F. H. et al., *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. et al., *Virology* 175:535, 1990),
- 25 particles of viral or synthetic origin (e.g., Kofler, N. et al., *J. Immunol. Methods.* 192:25, 1996; Eldridge, J. H. et al., *Sem. Hematol.* 30:16, 1993; Faló, L. D., Jr. et al., *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. et al., *Vaccine* 11:293, 1993), liposomes (Reddy, R. et al., *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or,
- 30 naked or particle absorbed cDNA (Ulmer, J. B. et al., *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. et al., In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. et al., *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor

mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptide(s). The peptide(s) can be individually linked to its own carrier; alternatively, the peptide(s) can exist as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune response. The composition may be a naturally occurring region of an antigen or may be prepared, *e.g.*, recombinantly or by chemical synthesis.

Furthermore, useful carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS).

As disclosed in greater detail herein, upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some instances it may be desirable to combine the class I peptide vaccines of the invention with vaccines which induce or facilitate neutralizing antibody responses to the target antigen of interest, particularly to viral envelope antigens. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I

and/or class II epitope in accordance with the invention, along with a PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142). Furthermore, any of these embodiments can be administered as a nucleic acid mediated modality.

- 5 The vaccine compositions of the invention may also be used in combination with antiviral drugs such as interferon- α .

- For therapeutic or prophylactic immunization purposes, the peptides of the invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus, for example, as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into an acutely or chronically infected host or into a non-infected host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g. adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.
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- Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. The resulting CTL or HTL cells, can be used to treat chronic infections, or tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular antigen (infectious or tumor-associated antigen) are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 14 weeks), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells. Alternatively, dendritic cells are transfected, e.g., with a minigene construct in accordance
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with the invention, in order to elicit immune responses. Minigenes will be discussed in greater detail in a following section.

Vaccine compositions may also be administered *in vivo* in combination with dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

- 5 DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") delivery.

- Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. Exemplary epitopes that may be utilized in a vaccine to treat or prevent HCV infection are set out in Tables XXVI-XXIX, and Table XXXII. It is preferred that each of the following principles are balanced in order to make the selection.

- 1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with HCV clearance. For HLA Class I this includes 3-4 epitopes that come from at least one antigen of HCV. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one HCV antigen (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450).

- 2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

- 3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth, or redundancy of, population coverage.

- 4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes. Of particular relevance for infectious disease vaccines

(but for cancer-related vaccines as well), are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

- 5 When providing nested epitopes, it is preferable to provide a sequence that has the greatest number of epitopes per provided sequence. Preferably, one avoids providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, it is important to
- 10 screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

- 5.) When creating a minigene, as disclosed in greater detail in the following section, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those
- 15 employed when selecting a peptide comprising nested epitopes. Furthermore, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is an actual binding epitope, as predicted, *e.g.*, by motif analysis, that only exists because two discrete peptide sequences are encoded directly
- 20 next to each other. Junctional epitopes are generally to be avoided because the recipient may generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

- Polyepitopic vaccine compositions may include epitopes from the core, S, E1,
- 25 NS1/E2, NS2, NS3, NS4, and NS5 domains of the HCV polyprotein. These regions encompass the following amino acid sequences using numbering relative to the prototype HCV-1 strain (Genbank accession number M62321; *see, e.g.*, US Patent Nos. 5,683,864 and 5,670,153): C domain (amino acids 1-120); S (amino acids 120-400); NS3 (amino acids 1050-1640); NS4 (amino acids 1640-2000); NS5 (amino acids 2000-3011); and
- 30 envelop proteins, E1 and E2/NS1, encompassing amino acids 192-750. Amino acids 750 to 1050 are designated as domain X as applied to the present invention. As appreciated by one of ordinary skill in the art, the designation of the amino acid range for each domain may diverge to some extent from that of HCV-1 depending on the strain of HCV.

One of ordinary skill in the art, when looking at an HCV polyprotein sequence, would readily be able to determine the domain boundaries.

- Specific embodiments of the polypeptidic compositions of the present invention include a pharmaceutical composition comprising a pharmaceutically acceptable carrier and combination of motif-bearing peptides that are immunologically cross-reactive with peptides of HCV-1, wherein at least one of the peptides bears a motif of Table Ia, and further wherein the combination of motif-bearing peptides consists of: a) one or more peptides comprising at least 8 amino acids from an HCV C domain; b) one or more peptides comprising at least 8 amino acids of a further domain selected from the group consisting of: an S domain, an NS3 domain, an NS4 domain, or an NS5 domain, and; c) optionally, one or more motif-bearing peptides from one or more additional HCV domains with a *proviso* that an additional domain is not a further domain listed in "b". Preferably, such a pharmaceutical composition may additionally comprise one or more distinct HCV motif-bearing peptide(s) comprising at least 8 amino acids of an X domain or, alternatively, the composition may further comprise additional HCV motif-bearing peptide(s) that are from an envelope domain, the envelope domain peptide(s) consisting of one or more copies of a single HCV peptide comprising at least 8 amino acids of an envelope domain.

- In another embodiment, the polypeptidic pharmaceutical composition may comprise a pharmaceutically acceptable carrier and combination of motif-bearing peptides that are immunologically cross-reactive with HCV-1 peptides, the peptides from multiple domains of HCV, wherein at least one of the peptides bears a motif of Table Ia, and wherein the combination of motif-bearing peptides consists essentially of: a) one or more peptides comprising at least 8 amino acids from a C domain; and, b) one or more peptides comprising at least 8 amino acids from an S, NS3, NS4, or NS5 domain, and, one HCV peptide comprising at least 8 amino acids of an envelope domain. Such a composition may further comprise one or more HCV motif-bearing peptides comprising at least 8 amino acids of an X domain.

- Alternatively, a pharmaceutical composition of the invention may comprise: a) a pharmaceutically acceptable carrier; and, b) a combination of one or more motif-bearing peptides of at least 8 amino acids derived from one or more hepatitis C virus (HCV) domains, wherein said peptides are cross-reactive with peptides of HCV-1, with a *proviso* that the combination does not include a peptide of at least 8 amino acids from an HCV C domain, and wherein at least one of the peptides bears a motif of Table Ia, said domains

selected from the group consisting of: an S domain; an NS3 domain; an NS4 domain; an NS5 domain; and, an X domain. Such a composition may additionally comprise motif-bearing HCV envelope peptide(s) consisting of one or more copies of a single HCV peptide comprising at least 8 amino acids of an envelope domain.

- 5 Lastly, an embodiment of the invention may comprise a pharmaceutical composition comprising a pharmaceutically acceptable carrier and combination of two or more motif-bearing peptides from a single domain of an HCV-1 strain, said peptides immunologically cross-reactive with peptides of an HCV-1 antigen, wherein at least one of the peptides bears a motif of Table Ia, and the peptides are derived from HCV, and the HCV domain is selected from the group consisting of: a C domain; an S domain; an NS3 domain; an NS4 domain; an NS5 domain; an X domain; or, an envelope domain from a single HCV strain, with a *proviso* that the envelope domain is other than a variable envelope domain.
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- 15 In the embodiments set forth, "peptides immunologically cross-reactive with HCV-1" refers to peptides that are bound by the same antibody; "derived from" refers to a fragment or subsequence and conservatively modified variants thereof.

IV.K.1. Minigene Vaccines

- 20 A growing body of experimental evidence demonstrates that a number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention. The use of multi-epitope minigenes is described below and in, *e.g.*, co-pending application U.S.S.N. 09/311,784; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding nine dominant HLA-A*0201- and A11-restricted epitopes derived from the polymerase, envelope, and core proteins of HBV and human immunodeficiency virus (HIV), the PADRE™ universal helper T cell (HTL) epitope, and an endoplasmic reticulum-translocating signal sequence was engineered. Immunization of HLA transgenic mice with this plasmid construct
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resulted in strong CTL induction responses against the nine epitopes tested, similar to those observed with a lipopeptide of known immunogenicity in humans, and significantly greater than immunization in oil-based adjuvants. Moreover, the immunogenicity of DNA-encoded epitopes *in vivo* correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these data show that the minigene served to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes. A similar approach may be used to develop minigenes encoding HCV epitopes.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus

(hCMV) promoter. See, e.g., U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (e.g., IL-2, IL-12, GM-CSF), cytokine-inducing molecules (e.g., LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (e.g. TGF- β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor

according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

- 5 Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for
- 10 formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids can also be used in the formulation (see, e.g., as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, glycolipids, fusogenic
- 15 liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

- 20 Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be
- 25 co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytotoxicity, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes may be evaluated in an analogous manner using assays to assess HTL
- 30 activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (e.g., IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA).

Twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ^{51}Cr -labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

IV.K.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising the peptides of the present invention, or analogs thereof, which have immunostimulatory activity may be modified to provide desired attributes, such as improved serum half life, or to enhance immunogenicity.

For instance, the ability of the peptides to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL epitopes to enhance immunogenicity is illustrated, for example, in co-pending U.S.S.N. 08/820360, U.S.S.N. 08/197,484, and U.S.S.N. 08/464,234.

Particularly preferred CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide may be linked to the T helper peptide without a spacer.

The CTL peptide epitope may be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated. The HTL peptide epitopes used in the invention can be modified in the same

manner as CTL peptides. For instance, they may be modified to include D-amino acids or be conjugated to other molecules such as lipids, proteins, sugars and the like. Exemplary T helper peptides include tetanus toxoid 830-843, influenza 307-319, and malarial circumsporozoite 382-398 and 378-398.

- 5 In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of amino acid sequences that are promiscuous include sequences from antigens
10 such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVNS), and Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

- Alternatively, it is possible to prepare synthetic peptides capable of stimulating T
15 helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (see, e.g., PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (e.g., PADRE™, Epimmune, Inc., San Diego, CA) are designed to most preferably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAAa,
20 where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and a is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

- 25 HTL peptide epitopes can also be modified to alter their biological properties. For example, peptides comprising HTL epitopes can contain D-amino acids to increase their resistance to proteases and thus extend their serum half-life. Also, the epitope peptides of the invention can be conjugated to other molecules such as lipids, proteins or sugars, or any other synthetic compounds, to increase their biological activity. Specifically, the T
30 helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes cytotoxic T

lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ϵ - and α -amino groups of a lysine residue and then linked, *e.g.*, via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.*, incomplete Freund's adjuvant. In a preferred embodiment, a particularly effective immunogenic comprises palmitic acid attached to ϵ - and α -amino groups of Lys, which is attached via linkage, *e.g.*, Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylserine (P₃CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide. (*See, e.g., Deres, et al., Nature* 342:561, 1989). Peptides of the invention can be coupled to P₃CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P₃CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

As noted herein, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide, particularly class I peptides. However, it is to be noted that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH₂ acylation, *e.g.*, by alkanoyl (C₁-C₂₀) or thiolglycolyl acetylation, terminal-carboxyl amidation, *e.g.*, ammonia, methylamine, *etc.* In some instances these modifications may provide sites for linking to a support or other molecule.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are useful for administration to mammals, particularly

humans, to treat and/or prevent HCV infection. Vaccine compositions containing the peptides of the invention are administered to a patient infected with HCV or to an individual susceptible to, or otherwise at risk for, HCV infection to elicit an immune response against HCV antigens and thus enhance the patient's own immune response capabilities. In therapeutic applications, peptide and/or nucleic acid compositions are administered to a patient in an amount sufficient to elicit an effective CTL and/or HTL response to the virus antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

The vaccine compositions of the invention may also be used purely as prophylactic agents. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg . Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 μg to about 50,000 μg of peptide administered at defined intervals from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine may be assessed by measuring the specific activity of CTL and HTL obtained from a sample of the patient's blood.

As noted above, peptides comprising CTL and/or HTL epitopes of the invention induce immune responses when presented by HLA molecules and contacted with a CTL or HTL specific for an epitope comprised by the peptide. The manner in which the peptide is contacted with the CTL or HTL is not critical to the invention. For instance, the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other vehicles, *e.g.*, DNA vectors encoding one or more peptides, viral vectors encoding the peptide(s), liposomes and the like, can be used, as described herein.

For pharmaceutical compositions, the immunogenic peptides of the invention, or DNA encoding them, are generally administered to an individual already infected with HCV. The peptides or DNA encoding them can be administered individually or as fusions of one or more peptide sequences. Those in the incubation phase or the acute

phase of infection can be treated with the immunogenic peptides separately or in conjunction with other treatments, as appropriate.

For therapeutic use, administration should generally begin at the first diagnosis of HCV infection. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. In chronic infection, loading doses followed by boosting doses may be required.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals. For those individuals susceptible (or predisposed) to developing chronic infection, the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. Where susceptible individuals are identified prior to or during infection, the composition can be targeted to them, thus minimizing the need for administration to a larger population.

The peptide or other compositions used for the treatment or prophylaxis of HCV infection can be used, *e.g.*, in persons who have not manifested symptoms of disease but who act as a disease vector. In this context, it is generally important to provide an amount of the peptide epitope delivered by a mode of administration sufficient to effectively stimulate a cytotoxic T cell response; compositions which stimulate helper T cell responses can also be given in accordance with this embodiment of the invention.

The dosage for an initial therapeutic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg . Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Boosting dosages of between about 1.0 μg to about 50000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood. The peptides and compositions of the present invention may be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

Thus, for treatment of chronic infection, a representative dose is in the range disclosed above, namely where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg , preferably from about 500 μg to about 50,000 μg per 70 kilogram patient. Initial doses followed by boosting doses at
 5 established intervals, *e.g.*, from four weeks to six months, may be required, possibly for a prolonged period of time to effectively immunize an individual. In the case of chronic infection, administration should continue until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted
 10 in accordance with methodologies known in the art.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral, intrathecal, or local administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides
 15 compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The
 20 resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium
 25 acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes,
 30 viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see*,

e.g., Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or to target selectively to infected cells, as well as to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the peptide compositions. Liposomes for use in accordance with the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of

peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

IV.M. Kits

The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form together with instructions for administration. Lymphokines such as IL-2 or IL-12 may also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

As in many viral diseases, there is evidence that clearance of HCV is mediated by CTL. In a study of primary HCV infection in six chimpanzees, four progressed to chronic infection (Cooper *et al.*, abstract, 19th US-Japan Hepatitis Joint Panel Meeting, January 27-29, 1998). It was found that these four animals showed either no CTL response or a very narrowly focused response during early infection. In contrast, in the remaining two animals that resolved the infection, a broad CTL response was observed against multiple HCV proteins, some of which were conserved. Weiner *et al.* (*Proc. Natl. Acad. Sci. USA* 92:2755-2759, 1995) demonstrated that viral escape, in which the

epitopes presented to PATR class I molecules mutated, was linked with a progression toward chronic infection. These data show a role for the CTL in directing the course of HCV disease, and in shaping the genetic composition of HCV species in the persistently infected host.

- 5 In work in humans, Koziel and co-workers have established the presence of HCV-specific CTL in liver infiltrates from patients with chronic HCV infection (Koziel *et al.*, *J. Immunol.* 149:3339, 1992; and Koziel *et al.*, *J. Virol.* 67:7522, 1993), and have also identified a number of CTL epitopes recognized in the context of several different HLA class I molecules. Other investigators have shown that HCV-specific CTL can be
10 detected in the peripheral blood of patients with chronic hepatitis C (Cerny *et al.*, *J. Clin. Invest.* 95:521, 1995; Cerny *et al.*, *Curr. Topics in Micro. and Immunol* 189:169, 1994; Cerny *et al.*, Abst. 2nd International Meeting on Hepatitis C and Related Viruses; La Jolla, CA, 1994; Battegay *et al.*, Abst. 2nd International Meeting on Hepatitis C and Related Viruses; La Jolla, CA, 1994; Shirai *et al.*, *J. Virol.* 68:3334, 1994; Shirai *et al.*, *J.*
15 *Immunol.* 154:2733, 1995; Battegay *et al.*, *J. Virol.* 69:2462, 1995). In addition, escape variants have been demonstrated in patients chronically infected with HCV (Chang *et al.*, *J. Clin. Invest.* 100:2376-2385, 1997; Tsai *et al.*, *Gastroenterology* 115:954-966, 1998).

- The magnitude of the CTL responses observed in HCV-infected patients is, in general, higher than those observed in the case of chronic hepatitis B infection,
20 suggesting that there is less impairment of specific T cell immunity than with HBV infection. The magnitude of CTL responses in HCV patients is, however, lower than those observed in HBV infected individuals who successfully cleared HBV infection. These results support the understanding that HCV infected patients are capable of responding to active immunotherapy, and suggest that potentiation and increasing of T
25 cell responses to HCV may be of use in therapy and prevention of chronic HCV infection (Prince, A. M. *FEMS Micro. Rev.* 14:273, 1994).

- Several groups have analyzed the potential role of HCV-specific CTL responses in disease resistance and pathogenesis. In some studies no correlation was found between CTL viremia and CTL precursor frequency for individual HCV epitopes (Rehermann *et al.*, *J. Clin. Invest.* 98:1432-1440, 1996; Wong *et al.*, *J. Immunol.* 160:1479-1488, 1998).
30 In other studies, however, it was shown that a clear correlation existed between levels of HCV infection and CTL responses, provided that the global response against multiple CTL epitopes was considered (Rehermann *et al.*, *J. Virol.* 70:7092-7102, 1996). These data represent a strong rationale for development of vaccine constructs capable of

inducing vigorous CTL responses directed against a multiplicity of conserved HCV-derived epitopes.

Koziel and colleagues have demonstrated the presence of HCV-specific CTLs, as well as T helper cell responses, in exposed but seronegative individuals (Koziel *et al.*, *J. Infect. Diseases* 176:859-866, 1997). In addition, HCV-specific CTLs have been detected in healthy, seronegative family members of chronically HCV-infected patients, indicating that a protective immunity is established in absence of a detectable infection (Bronowicki *et al.*, *J. Infect. Dis.* 176:518-522, 1997; Scognamiglio *et al.*, in preparation).

Experimental evidence also indicates that HTL epitopes play an important role in immune reactivity and defenses against HCV infection (Missale *et al.*, *J. Clin. Invest.* 98:706-714, 1996). Diepolder *et al.* (in *Lancet* 346:1006, 1995) have shown that a region of the NS3 gene (NS3 1007-1534) is recognized by patients who clear acute HCV infection, but is not seen by patients who develop chronic infection. Subsequent studies have shown that this particular region contained a highly cross-reactive HTL epitope (NS3 1248-1261), which binds with good affinity to 10 of 13 DR molecules tested, and is highly conserved in 30/33 different HCV isolates considered (Diepolder *et al.*, *J. Virol.* 71:6011-6019, 1997). These data suggest that directing HTL responses to this type of epitope (rather than to less cross-reactive and/or highly variable ones) will be of therapeutic and prophylactic benefit and strongly argue for inclusion of this and other epitopes with similar characteristics in HCV vaccine constructs.

The following examples illustrate identification, selection, and use of immunogenic Class I and Class II peptide epitopes for inclusion in vaccine compositions.

Example 1: HLA Class I and Class II Binding Assays

The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

Epstein-Barr virus (EBV)-transformed homozygous cell lines, fibroblasts, CIR, or 721.22 transfectants were used as sources of HLA class I molecules. These cells were maintained *in vitro* by culture in RPMI 1640 medium supplemented with 2mM L-glutamine (GIBCO, Grand Island, NY), 50μM 2-ME, 100μg/ml of streptomycin, 100U/ml of penicillin (Irvine Scientific) and 10% heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). Cells were grown in 225-cm² tissue culture flasks or, for large-scale

cultures, in roller bottle apparatuses. The specific cell lines routinely used for purification of MHC class I and class II molecules are listed in Table XXIV.

Cell lysates were prepared and HLA molecules purified in accordance with disclosed protocols (Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al.*, *J. Immunol.* 154:247 (1995); Sette, *et al.*, *Mol. Immunol.* 31:813 (1994)). Briefly, cells were lysed at a concentration of 10^8 cells/ml in 50 mM Tris-HCl, pH 8.5, containing 1% Nonidet P-40 (Fluka Biochemika, Buchs, Switzerland), 150 mM NaCl, 5 mM EDTA, and 2 mM PMSF. Lysates were cleared of debris and nuclei by centrifugation at 15,000 x g for 30min.

HLA molecules were purified from lysates by affinity chromatography. Lysates prepared as above were passed twice through two pre-columns of inactivated Sepharose CL4-B and protein A-Sepharose. Next, the lysate was passed over a column of Sepharose CL-4B beads coupled to an appropriate antibody. The antibodies used for the extraction of HLA from cell lysates are listed in Table XXV. The anti-HLA column was then washed with 10-column volumes of 10mM Tris-HCL, pH 8.0, in 1% NP-40, PBS, 2-column volumes of PBS, and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, MHC molecules were eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0. Eluates were then be concentrated by centrifugation in Centriprep 30 concentrators at 2000 rpm (Amicon, Beverly, MA). Protein content was evaluated by a BCA protein assay (Pierce Chemical Co., Rockford, IL) and confirmed by SDS-PAGE.

A detailed description of the protocol utilized to measure the binding of peptides to Class I and Class II MHC has been published (Sette *et al.*, *Mol. Immunol.* 31:813, 1994; Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998). Briefly, purified MHC molecules (5 to 500nM) were incubated with various unlabeled peptide inhibitors and 1-10nM 125 I-radiolabeled probe peptides for 48h in PBS containing 0.05% Nonidet P-40 (NP40) (or 20% w/v digitonin for H-2 IA assays) in the presence of a protease inhibitor cocktail. The final concentrations of protease inhibitors (each from CalBioChem, La Jolla, CA) were 1 mM PMSF, 1.3 nM 1.10 phenanthroline, 73 μ M pepstatin A, 8mM EDTA, 6mM N-ethylmaleimide (for Class II assays), and 200 μ M N alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK). All assays were performed at pH 7.0 with the exception of DRB1*0301,

which was performed at pH 4.5, and DRB1*1601 (DR2w21 β ₁) and DRB4*0101 (DRw53), which were performed at pH 5.0. pH was adjusted as described elsewhere (see Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998).

- 5 Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration on 7.8 mm x 15 cm TSK200 columns (TosoHaas 16215, Montgomeryville, PA), eluted at 1.2 mls/min with PBS pH 6.5 containing 0.5% NP40 and 0.1% NaN₃. Because the large size of the radiolabeled peptide used for the DRB1*1501 (DR2w2 β ₁) assay makes separation of bound from unbound peaks more difficult under
- 10 these conditions, all DRB1*1501 (DR2w2 β ₁) assays were performed using a 7.8mm x 30cm TSK2000 column eluted at 0.6 mls/min. The eluate from the TSK columns was passed through a Beckman 170 radioisotope detector, and radioactivity was plotted and integrated using a Hewlett-Packard 3396A integrator, and the fraction of peptide bound was determined.

- 15 Radiolabeled peptides were iodinated using the chloramine-T method. Representative radiolabeled probe peptides utilized in each assay, and its assay specific IC₅₀ nM, are summarized in Tables IV and V. Typically, in preliminary experiments, each MHC preparation was titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of
- 20 the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

- Since under these conditions [label]<[HLA] and IC₅₀≥[HLA], the measured IC₅₀ values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μg/ml to 1.2 ng/ml, and are tested in
- 25 two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC₅₀ of a positive control for inhibition by the IC₅₀ for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values
- 30 can subsequently be converted back into IC₅₀ nM values by dividing the IC₅₀ nM of the positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for

comparing peptides that have been tested on different days, or with different lots of purified MHC.

- Because the antibody used for HLA-DR purification (LB3.1) is α -chain specific, β_1 molecules are not separated from β_3 (and/or β_4 and β_5) molecules. The β_1 specificity of the binding assay is obvious in the cases of DRB1*0101 (DR1), DRB1*0802 (DR8w2), and DRB1*0803 (DR8w3), where no β_3 is expressed. It has also been demonstrated for DRB1*0301 (DR3) and DRB3*0101 (DR52a), DRB1*0401 (DR4w4), DRB1*0404 (DR4w14), DRB1*0405 (DR4w15), DRB1*1101 (DR5), DRB1*1201 (DR5w12), DRB1*1302 (DR6w19) and DRB1*0701 (DR7). The problem of β chain specificity for DRB1*1501 (DR2w2 β_1), DRB5*0101 (DR2w2 β_2), DRB1*1601 (DR2w21 β_1), DRB5*0201 (DR51Dw21), and DRB4*0101 (DRw53) assays is circumvented by the use of fibroblasts. Development and validation of assays with regard to DR β molecule specificity have been described previously (*see, e.g., Southwood et al., J. Immunol.* 160:3363-3373, 1998).
- Binding assays as outlined above may be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

Example 2. Identification of Conserved HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

- Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif- and motif-bearing epitopes for the inclusion in such a vaccine composition. Calculation of population coverage was performed using the strategy described below.

Computer searches and algorithms for identification of supermotif and/or motif-bearing epitopes

- Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated HCV isolate sequences were analyzed using a text string search software program, *e.g., MotifSearch 1.4* (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs; alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be

made mentally. Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined motifs (that is, to account for the impact of different amino acids at different positions), and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$“\Delta G” = a_{1i} \times a_{2i} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide. This assumption is justified by studies from our laboratories that demonstrated that peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (see also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998).

Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i . For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

Complete polyprotein sequences from fourteen HCV isolates were aligned, then scanned, utilizing motif identification software, to identify conserved 9- and 10-mer sequences containing the HLA-A2-supermotif main anchor specificity.

A total of 231 conserved, HLA-A2 supermotif-positive sequences were identified. These peptides were then evaluated for the presence of A*0201 preferred secondary anchor residues using A*0201-specific polynomial algorithms. A total of 67 conserved, motif-bearing and algorithm-positive sequences were identified.

5 Fifty of these conserved, motif-containing 9- and 10-mer peptides were tested for their capacity to bind to purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule). Sixteen peptides bound A*0201 with IC₅₀ values ≤500 nM; 4 with high binding affinities (IC₅₀ values ≤50 nM) and 12 with intermediate binding affinities, in the 50-500 nM range (Table XXVI).

10 These 16 peptides were subsequently tested for the capacity to bind to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). As shown in Table XXVI, most of these peptides were found to be A2-supertype cross-reactive binders. More specifically, 12/16 (75%) peptides bound at least three of the five A2-supertype molecules tested.

15 *Selection of HLA-A3 supermotif-bearing epitopes*

The sequences from the same fourteen known HCV isolates scanned above were also examined for the presence of conserved peptides with the HLA-A3-supermotif primary anchors. A total of 71 conserved 9- or 10-mer motif containing sequences were identified. Further analysis using the A03 and A11 algorithms (see, e.g., Gulukota et al, *J. Mol. Biol.* 267:1258-1267, 1997 and Sidney et al, *Human Immunol.* 45:79-93, 1996) identified 39 sequences that scored high in either or both algorithms. Twenty seven of the 39 peptides were synthesized and tested for binding to HLA-A*03 and HLA-A*11, the two most prevalent A3-supertype molecules. Fifteen peptides were identified which bound A3 and/or A11 with binding affinities of ≤500 nM (Table XXVII). These peptides were then tested for binding cross-reactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801). Seven of the 15 peptides bound at least three of the five HLA-A3-supertype molecules tested.

30 In the course of an independent series of experiments (Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994), one peptide, HCV NS3 1262, not identified by the selection criteria utilized above because it does not have the A3-supermotif main anchor specificity, was determined to be cross-reactive in the A3-supertype, binding A*03, A*11, and A*6801. It is also shown in Table XXVII. Interestingly, this peptide

represents a single residue N-terminal truncation of peptide 1073.14, which is also shown in Table XXVII.

In summary, 8 peptides that bind 3 or more A3-supertype molecules derived from conserved regions of the HCV genome were identified.

Selection of HLA-B7 supermotif bearing epitopes

When the same fourteen HCV isolates were also analyzed for the presence of conserved 9- or 10-mer peptides with the HLA-B7-supermotif, 35 sequences were identified. The corresponding peptides were synthesized and tested for binding to HLA-B*0702, the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Thirteen peptides bound B*0702 with IC_{50} of ≤ 500 nM (Table XXVIIIa). These 13 peptides were then tested for binding to other common B7-supertype molecules (B*3501, B*51, B*5301, and B*5401). As shown in Table XXVIIIa, only 1 peptide (Core 169) was capable of binding to three or more of the five B7-supertype alleles tested.

To identify additional B7-supertype epitopes, further studies were undertaken. The protein sequences from the fourteen HCV isolates utilized above were again examined to identify conserved, motif-containing 8- and 11-mers. The isolates were also examined for 9- and 10-mer sequences allowing for lower conservancy (51%-78%). These analyses identified twenty-five 8-mers, sixteen 11-mers, and thirty-five 9- and 10-mers. These peptides were synthesized and tested for binding to B*0702. Thirteen peptides bound with high or intermediate affinity to B*0702 ($IC_{50} \leq 500$ nM) (Table XXVIIIb). These peptides were additionally screened for binding to other B7-supertype molecules. Only one cross-reactive binder, the NS3 1378 8-mer (peptide 29.0035/1260.04), was identified (Table XXVIIIb).

In summary, a total of two cross-reactive B7-supertype binders were identified (Core 169 and NS3 1378).

Selection of A1 and A24 motif-bearing epitopes

To further increase population coverage, HLA-A1 and -A24 epitopes can also be incorporated into potential vaccine constructs.

In a previous analysis, two A1 and three A24 binders, 100% conserved among four strains of HCV, were identified (Wentworth *et al.*, *Int. Immunol.* 8:651-659, 1996). An analysis of the protein sequence data from the fourteen HCV strains utilized above

demonstrated that these peptides were >79% conserved, and also identified an additional eleven A1- and twenty five A24-motif-containing conserved sequences (see Table XXIXA and B). Testing for binding to the appropriate HLA molecule (*i.e.*, A1 or A24) was completed for eight of the additional eleven A1 peptides, and seven of the additional
 5 twenty five A24 peptides. Overall, as shown in Table XXIX, four A1-motif peptides (A) and three A24-motif peptides (B) have been found with binding capacities of 500 nM or less for the appropriate allele-specific HLA molecule.

Analysis of the HLA-A2 and A3 supermotif-bearing epitopes identified above revealed that in 13/14 cases, peptides binding the supertype prototype HLA molecule (*i.e.*
 10 A*0201 for the A2 supertype, and A*0301 for the A3 supertype) with an IC_{50} of less than 100nM were cross-reactive and recognized by HCV-infected patients as described in Example 3, which follows. Based on these observations, two A1 peptides and one A24 peptide epitopes were also selected as candidates for inclusion in vaccine compositions; these peptides bind the appropriate HLA molecule with an IC_{50} of less than 100nM.

Example 3: Confirmation of Immunogenicity

Evaluation of A*0201 immunogenicity

It has been shown that CTL induced in A*0201/K^b transgenic mice exhibit specificity similar to CTL induced in the human system (*see, e.g.*, Vitiello *et al.*, *J. Exp.*
 20 *Med.* 173:1007-1015, 1991; Wentworth *et al.*, *Eur. J. Immunol.* 26:97-101, 1996). Accordingly, these mice were used to evaluate the immunogenicity of the twelve conserved A2-supertype cross-reactive peptides identified in Example 2 above.

CTL induction in transgenic mice following peptide immunization has been described (Vitiello *et al.*, *J. Exp. Med.* 173:1007-1015, 1991; Alexander *et al.*; *J.*
 25 *Immunol.* 159:4753-4761, 1997). In these studies, mice were injected subcutaneously at the base of the tail with each peptide (50 μ g/mouse) emulsified in IFA in the presence of an excess of an IA^b-restricted helper peptide (140 μ g/mouse) (HBV core 128-140, Sette *et al.*, *J. Immunol.* 153:5586-5592, 1994). Eleven days after injection, splenocytes were incubated in the presence of peptide-loaded syngenic LPS blasts. After six days, cultures
 30 were assayed for cytotoxic activity using peptide-pulsed targets. The data, summarized in Table XXX, indicate that 7 of the 12 peptides (58%) were capable of inducing primary CTL responses in A*0201/K^b transgenic mice. (For these studies, a peptide was

considered positive if it induced CTL (L.U. $30/10^6$ cells ≥ 2 in at least two transgenic animals (Wentworth *et al.*, *Eur. J. Immunol.* 26:97-101, 1996).

The conserved, cross reactive candidate CTL epitopes were also tested for recognition *in vitro* by PBMCs obtained from HCV-infected patients. Briefly, PBMC from patients infected with HCV were cultured in the presence of 10 $\mu\text{g/ml}$ of synthetic peptide. After 7 and 14 days, the cultures were restimulated with peptide. The cultures were assayed for cytolytic activity on day 21 using target cells pulsed with the specific peptide in a standard four hour ^{51}Cr release assay. The data are summarized in Table XXX. As shown, all 12 peptides are CTL epitopes recognized by PBMC from HCV-infected patients. From the data in Table XXX, it is interesting to note that HLA transgenics did not fully reveal the immunogenicity of some peptides that were positive in recall responses. This apparent discrepancy may reflect differences in the route of immunization utilized (*e.g.*, natural infection versus peptide immunization), or CTL repertoire.

*Evaluation of A*03/A11 immunogenicity*

The immunogenicity of six of the eight A3-supertype cross-reactive peptides identified in Example 2 above was evaluated in HLA-A11/K^b transgenic mice, using the protocol described above for HLA-A2 transgenic mice (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). Five of these six peptides were able to induce primary CTL responses (Table XXXI).

All eight peptides were also studied by collaborators using PBMC cultures from HCV infected patients and contacts of such patients. This data is also summarized in Table XXXI. Briefly, all eight peptides were recognized by HCV infected individuals.

Evaluation of B7 immunogenicity

One of the two B7-supertype cross-reactive peptides (1145.12, Core 169) has been evaluated for immunogenicity in HCV-infected patients. Two independent collaborators have shown that this peptide is indeed immunogenic, and is recognized by T cells from HCV-infected patients (Chang *et al.*, *J. Immunol.* 162:1156-1164, 1999)

Example 4: Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analogued, or “fixed” to confer upon the peptide certain characteristics, *e.g.* greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analog peptides that exhibit modulated binding affinity are set forth in this example.

Analoging at Primary Anchor Residues

As shown in Example 2, more than ten different HCV-derived, A2-supertype-restricted epitopes were identified. Peptide engineering strategies are implemented to further increase the cross-reactivity of the candidate epitopes identified above which bind 3/5 of the A2 supertype alleles tested. On the basis of the data disclosed, *e.g.*, in related and co-pending U.S.S.N 09/226,775, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

To analyze the cross-reactivity of the analog peptides, each engineered analog is initially tested for binding to the prototype A2 supertype allele A*0201, then, if A*0201 binding capacity is maintained, for A2-supertype cross-reactivity.

Similarly, analogs of HLA-A3 supermotif-bearing epitopes may also be generated. For example, peptides binding to 3/5 of the A3-supertype molecules may be engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2.

The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate ≤ 500 nM binding capacity are then tested for A3-supertype cross-reactivity.

Similarly to the A2- and A3- motif bearing peptides, peptides binding 3 or more B7-supertype alleles may be improved, where possible, to achieve increased cross-reactive binding. B7 supermotif-bearing peptides may, for example, be engineered to

possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position, as demonstrated by Sidney *et al.* (*J. Immunol.* 157:3480-3490, 1996).

Analoging at Secondary Anchor Residues

- 5 Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying particular residues at secondary anchor positions that are associated with such properties. Demonstrating this, the binding capacity of a peptide representing a discreet single amino acid substitution at position one was analyzed. Peptide 1145.13 (Table XXVIIIc), which
10 represents the substitution of L to F at position 1 of the core 169 sequence, binds all five B7-supertype molecules with a good affinity (all IC₅₀ values \leq 132 nM), and in 3 instances has higher affinity over that of the parent peptide by >35-fold.

- Because so few B7-supertype cross-reactive epitopes were identified, our results from previous binding evaluations were analyzed to identify conserved (8-, 9-, 10-, or 11-
15 mer) peptides which bind, minimally, 3/5 B7 supertype molecules with weak affinity (IC₅₀ of 500nM-5 μ M). This analysis identified 9 peptides, 6 of which are analogued (including core 169 which had been previously analogued). These peptides are tested for enhanced binding affinity and B7-supertype cross-reactivity.

- Engineered analogs with sufficiently improved binding capacity or cross-
20 reactivity are tested for immunogenicity in HLA-B7-transgenic mice, following for example, IFA immunization or lipopeptide immunization.

- In conclusion, these data demonstrate that by the use of even single amino acid substitutions, it is possible to increase the binding affinity and/or cross-reactivity of peptide ligands for HLA supertype molecules.

- 25 Example 5: Identification of conserved HCV-derived sequences with HLA-DR binding motifs

- Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

- 30 *Selection of HLA-DR-supermotif-bearing epitopes*

 To identify HCV-derived, HLA class II HTL epitopes, the same fourteen HCV polyprotein sequences used for the identification of HLA Class I supermotif/motif

sequences were analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif. Specifically, 15-mer sequences were selected comprising a DR-supermotif, further comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total). It was also required that the 15-mer sequence be conserved in at least 79% (11/14) of the HCV strains analyzed. These criteria identified a total of 49 non-redundant sequences, which are shown in Table XXXIIA. (In the context of Class II epitopes, a sequence is considered operationally redundant if more than 80% of it's sequence overlaps with another peptide.)

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele specific selection tables (see, *e.g.*, Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select peptide sequences with a high probability of binding a particular DR molecule. Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

To see if these protocols serve to identify additional epitopes, the same HCV polypeptides used above were re-scanned for the presence of 15-mer peptides with 9-mer core regions that were $\geq 79\%$ (11/14 strains) conserved. This identified 152 sequences; 49 of which were identified previously, as described above. Next, the 9-mer core region of each of these peptides was scored using the DR1, DR4w4, and DR7 algorithms. Twenty-two peptides, including 12 new sequences (10 peptides were from the original set of 49) were found to have 9-mer cores with protocol-derived scores predictive of cross-reactive DR binders. The 12 additional sequences are shown in Table XXXIIB.

The conserved, HCV-derived peptides identified above were tested for their binding capacity for various common HLA-DR molecules. All peptides were initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least 2 of these 3 DR molecules were then tested for binding to DR2w2 β 1, DR2w2 β 2, DR6w19, and DR9 molecules in secondary assays. Finally, peptides binding at least 2 of the 4 secondary panel DR molecules, and thus cumulatively at least 4 of 7 different DR molecules, were screened for binding to DR4w15, DR5w11,

and DR8w2 molecules in tertiary assays. Peptides binding at least 7 of the 10 DR molecules comprising the primary, secondary, and tertiary screening assays were considered cross-reactive DR binders. The composition of these screening panels, and the phenotypic frequency of associated antigens, are shown in Table XXXIII.

5 Upon testing, it was found that 29 of the original 75 peptides (39%) bound two or more of the primary HLA molecules. Twenty-six of these cross-reactive binders were then tested in the secondary assays, and nineteen were found to bind at least four of the seven HLA DR molecules in the primary and secondary panels. Finally, the nineteen peptides passing the secondary screening phase were tested for binding in the tertiary
10 assays. As a result, nine peptides were identified which bound at least seven of ten common HLA-DR molecules. Table XXXIV shows these nine peptides and their binding capacity for each allele-specific HLA-DR molecule in the primary through tertiary panels. Also shown in Table XXXIV are two peptides (F134.05 and F134.08) for which a complete binding analysis was not performed. However, both of these peptides bound six
15 of the seven HLA DR molecules tested. F134.08 nests peptide 1283.44, which bound eight of 10 allele-specific HLA molecules.

In conclusion, eleven cross-reactive DR-binding peptides, derived from six discrete (*i.e.* non-redundant) regions of the HCV genome, have been identified. Two of the six regions from which these epitopes were derived are covered by multiple,
20 overlapping epitopes.

Selection of conserved DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL
25 epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts with other DR alleles (Sidney *et al.*, *J. Immunol.* 149:2634-2640, 1992; Geluk *et al.*, *J. Immunol.* 152:5742-5748, 1994; Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles.

30 To efficiently identify peptides that bind DR3, target proteins were analyzed for conserved sequences carrying one of the two DR3 specific binding motifs reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). Fifteen sequences, including a peptide nested within a DR-supermotif sequence identified above (peptide Pape 22), were

identified (Table XXXIId). Preferably, DR3 motifs will be found clustered in proximity with DR supermotif regions.

- Fourteen of the fifteen peptides containing a DR3 motif were tested for their DR3 binding capacity. Two peptides (CH35.0106 and CH35.0107) were found to bind DR3 with an affinity of $1\mu\text{M}$ or less (Table XXXV), and thereby qualify as HLA class II high affinity binders.

DR3 binding epitopes identified in this manner may then be included in vaccine compositions with DR supermotif-bearing peptide epitopes.

10 Example 6: Immunogenicity of candidate HCV-derived HTL epitopes and known dominant HCV HTL epitope

In the course of collaborative studies with G. Pape and C. Ferrari, eight conserved, HCV-derived peptides have been identified which are recognized by HCV-infected individuals.

- One of these studies (Diepolder *et al.*, *J. Virol.* 71:6011-6019, 1997), identified peptide F98.05, which spans residues 1248-1261 of the NS3 protein, as an immunodominant CD4+ T-cell epitope that was recognized by 14/23 NS3-specific CD4+ T-cell clones from 4/5 patients with acute hepatitis C infection. This epitope, shown above to be an HLA-DR cross-reactive binder (see Table XXXIV), was capable of being presented to helper CD4+ T cells by multiple HLA molecules (DR4, DR11, DR12, DR13, and DR16). Two other peptides, Pape 22 and Pape 29, were also recognized by CD4+ T cell clones, although, in a more limited context; correspondingly, neither of these peptides are DR-cross-reactive binders.

- By direct peripheral blood T cell stimulation and by fine specificity analysis of HCV-specific T-cell lines and clones, studies done in collaboration with Ferrari's group identified 6 immunodominant epitopes, including one also identified in the Pape collaboration, that are derived from conserved regions of the core, NS3, and NS4 proteins. These epitopes were also found to be cross-reactive, being presented to T cells in the context of different Class II molecules. Three of the 6 epitopes, F98.04 (F134.03), F134.05 and F134.08, are cross-reactive HLA-DR binders (see Table XXXIV).

In conclusion, the immunogenicity of 8 epitopes derived from conserved regions of the HCV genome has been demonstrated. Three of these epitopes (F98.05, F134.05, and F134.08; see Table XXXIV) are broadly cross-reactive HLA-DR binding peptides.

Example 7. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae $gf=1-(\text{SQRT}(1-af))$ (see, e.g., Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula $[af=1-(1-Cgf)^2]$.

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and only alleles confirmed to belong to each of the supertypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (e.g., $\text{total}=A+B*(1-A)$). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801. Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An

analogous approach can be used to estimate population coverage achieved with combinations of class II motif-bearing epitopes.

Summary of candidate HLA class I and class II epitopes

- 5 In summary, on the basis of the data presented in the above examples, 26 CTL candidate peptide epitopes derived from conserved regions of the HCV virus have been identified (Table XXXVIa). These include twelve HLA-A2 supermotif-bearing epitopes, eight HLA-A3 supermotif-bearing epitopes, and one HLA-B7 supermotif-bearing epitope, each capable of binding to multiple A2-, A3-, or B7-supertype molecules, and
- 10 immunogenic in HLA transgenic mice or antigenic for human PBL (with the exception of peptide 29.0035/1260.04). Additional epitopes not evaluated for immunogenicity are also included. They are an additional B7-supermotif-bearing epitope and two HLA-A1 and one HLA-A24 high-affinity binding peptides. A known HLA-A31 restricted epitope (VGIYLLPNR), which also binds HLA-A33, is also set out in Table XXXVIa and is
- 15 useful in combination with other Class I or Class II epitopes.

- With these 26 CTL epitopes (as disclosed herein and from the art), average population coverage, (*i.e.*, recognition of at least one HCV epitope), is predicted to be greater than 95% in each of five major ethnic populations. The potential redundancy of coverage afforded by 25 of these epitopes (the peptide 24.0086 was not included) was
- 20 estimated using the game theory Monte Carlo simulation analysis, which is known in the art (see *e.g.*, Osborne, M.J. and Rubinstein, A. "A course in game theory" MIT Press, 1994). As shown in Figure 1, it is estimated that 90% of the individuals in a population comprised of the Caucasian, North American Black, Japanese, Chinese, and Hispanic ethnic groups would recognize 2 or more of the candidate epitopes described herein.

- 25 A list of HCV-derived HTL epitopes that would be preferred for use in the design of minigene constructs or other vaccine formulations is summarized in Table XXXVIb. As shown, 9 different peptide-binding regions have been identified which bind multiple HLA-DR molecules or bind HLA-DR3. (In the case of the NS4 1914-1935 region, the longer peptide, F134.08, recognized by patients, was chosen over the shorter peptide,
- 30 1283.44. The longer peptide essentially incorporates the shorter peptide, and also binds additional DR molecules that the shorter peptide does not bind.) Three of these peptides have been recognized as dominant epitopes in HCV infected patients.

It is estimated that each of 10 common DR molecules recognizing the DR supermotif, and DR3, are covered by a minimum of 2 epitopes. Correspondingly, the

total estimated population coverage represented by this panel of epitopes is in excess of 91% in each of the 5 major ethnic populations (Table XXXVII).

Example 8: Recognition Of Generation Of Endogenous Processed Antigens After

5 Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-6 recognize endogenously synthesized, *i.e.*, native antigens.

Effector cells isolated from transgenic mice that are immunized with peptide
10 epitopes as in Example 3, for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ⁵¹Cr labeled Jurkat-A2.1/K^b target cells in the absence or presence of
15 peptide, and also tested on ⁵¹Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* cells that are stably transfected with HCV expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized HCV antigen. The choice of transgenic mouse model to be used for such an analysis depends upon the epitope(s) that
20 is being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed, which may be used to evaluate HTL epitopes.

25

Example 9: Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice by use of an HCV CTL/HTL peptide conjugate whereby the vaccine composition comprises peptides administered to an HCV-infected patient or an individual at risk for HCV. The
30 peptide composition can comprise multiple CTL and/or HTL epitopes. This analysis demonstrates enhanced immunogenicity that can be achieved by inclusion of one or more HTL epitopes in a vaccine composition. Such a peptide composition can comprise a lipidated HTL epitope conjugated to a preferred CTL epitope containing, for example, at

least one CTL epitope selected from Table XXVI-XXIX, or an analog of that epitope. The HTL epitope is, for example, selected from Table XXXII.

Lipopeptide preparation: Lipopeptides are prepared by coupling the appropriate fatty acid to the amino terminus of the resin bound peptide. A typical procedure is as follows: A dichloromethane solution of a four-fold excess of a pre-formed symmetrical anhydride of the appropriate fatty acid is added to the resin and the mixture is allowed to react for two hours. The resin is washed with dichloromethane and dried. The resin is then treated with trifluoroacetic acid in the presence of appropriate scavengers [e.g. 5% (v/v) water] for 60 minutes at 20°C. After evaporation of excess trifluoroacetic acid, the crude peptide is washed with diethyl ether, dissolved in methanol and precipitated by the addition of water. The peptide is collected by filtration and dried.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are useful for the assessment of the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-bearing epitopes, are primed subcutaneously (base of the tail) with 0.1 ml of peptide conjugate formulated in saline, or DMSO/saline. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

Cell lines: Target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (*e.g.*, Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991)

In vitro CTL activation: One week after priming, spleen cells (30×10^6 cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts (10×10^6 cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to 1.5×10^6) are incubated at 37°C in the presence of 200 μ l of ^{51}Cr . After 60 minutes, cells are washed three times and resuspended in R10 medium. Peptide is added where required at a concentration of 1 μ g/ml. For the assay, 10^4 ^{51}Cr -labeled target cells are added to different concentrations of effector cells (final volume of 200 μ l) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = $100 \times$

(experimental release - spontaneous release)/(maximum release - spontaneous release).

To facilitate comparison between separate CTL assays run under the same conditions, % ^{51}Cr release data is expressed as lytic units/ 10^6 cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ^{51}Cr release assay. To obtain specific lytic units/ 10^6 , the lytic units/ 10^6 obtained in the absence of peptide is subtracted from the lytic units/ 10^6 obtained in the presence of peptide. For example, if 30% ^{51}Cr release is obtained at the effector (E): target (T) ratio of 50:1 (i.e., 5×10^5 effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., 5×10^4 effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000)-(1/500,000)] \times 10^6 = 18 \text{ LU}$.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immunogenic CTL/HTL conjugate vaccine preparation and are compared to the magnitude of the CTL response achieved using the CTL epitope as outlined in Example 3. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is induced, and concomitantly that an HTL response is induced upon administration of such compositions.

20 Example 10. Selection of CTL and HTL epitopes for inclusion in an HCV-specific vaccine.

This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention. The peptides in the composition may be in the form of a nucleic acid sequence, either single or one or more sequences (i.e., minigene) that encodes peptide(s), or may be single and/or polypeptidic peptides.

The following principles are utilized when selecting an array of epitopes for inclusion in a vaccine composition. Each of the following principles are balanced in order to make the selection.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with HCV clearance. For HLA Class I this includes 3-4 epitopes that come from at least one antigen of HCV. In other words, it has been observed that patients who spontaneously clear HCV generate an immune response

to at least 3 epitopes on at least one HCV antigen. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one HCV antigen.

- 2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

- 3.) Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art and discussed herein, can be employed to assess breadth, or redundancy, of population coverage.

- 4.) When selecting epitopes for HCV antigens it may be preferable to select native epitopes. Therefore, of particular relevance for infectious disease vaccines, are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

- When providing nested epitopes, a sequence that has the greatest number of epitopes per provided sequence is provided. A limitation on this principle is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, the sequence is screened in order to insure that it does not have pathological or other deleterious biological properties.

- 5.) When creating a minigene, as disclosed in greater detail in Example 11, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is an actual binding epitope, as predicted, *e.g.*, by motif analysis. Junctional epitopes are generally to be avoided because the recipient may generate an immune response to that epitope, which is not present in a native HCV protein sequence. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

Peptide epitopes for inclusion in vaccine compositions are, for example, selected from those listed in Tables XXVI-XXIX and Table XXXII. A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response similar in magnitude of an immune response that clears an acute HCV infection.

Example 11: Construction of Minigene Multi-Epitope DNA Plasmids

This example provides general guidance for the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL and/or HTL epitopes or epitope analogs as described herein. Expression plasmids have been constructed and evaluated as described, for example, in co-pending U.S.S.N. 09/311,784 filed 5/13/99. An example of such a plasmid for the expression of HCV epitopes is shown in Figure 2, which illustrates the orientation of HCV peptide epitopes in a minigene construct.

A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes (Figure 2). Preferred epitopes are identified, for example, in Tables XXVI-XXIX and XXXII. HLA class I supermotif or motif-bearing peptide epitopes derived from multiple HCV antigens, *e.g.*, the core, NS4, NS3, NS5, NS1/E2, are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple HCV antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector.

Overlapping oligonucleotides, for example eight oligonucleotides, averaging approximately 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final

5 multipeptide minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated T_m of each primer pair) for 30 sec, and 72°C for 1 min.

10 For the first PCR reaction, 5 µg of each of two oligonucleotides are annealed and extended: Oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and

15 two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product for 25 additional cycles. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by

20 sequencing.

Example 12. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which the plasmid construct prepared using the methodology outlined in Example 11 is able to induce immunogenicity is evaluated through *in vivo*

25 injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analysed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.*, in U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994. To assess the capacity of the pMin minigene construct to induce CTLs *in vivo*, HLA-A11/K^b transgenic mice, for example, are immunized intramuscularly with 100 µg of naked

30 cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ^{51}Cr release assay. The results indicate the magnitude of the CTL response directed against the A3-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A3 supermotif peptide epitopes as does the polyepitopic peptide vaccine. A similar analysis is also performed using other HLA-A2 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A2 and HLA-B7 motif or supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, I-A^b restricted mice, for example, are immunized intramuscularly with 100 μg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant.

CD4⁺ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is measured using a ^3H -thymidine incorporation proliferation assay, (*see, e.g.,* Alexander et al. Immunity 1:751-761, 1994). the results indicate the magnitude of the HTL response, thus demonstrating the *in vivo* immunogenicity of the minigene.

Example 13: Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent HCV infection in persons who are at risk for such infection. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to individuals at risk for HCV infection. The composition is provided as a single lipidated polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freund's Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 μg , generally 100-5,000 μg , for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by

techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against HCV infection.

- Alternatively, the polyepitopic peptide composition can be administered as a
 5 nucleic acid in accordance with methodologies known in the art and disclosed herein.

Example 14: Polyepitopic Vaccine Compositions Derived from Native HCV Sequences

- A native HCV polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify
 10 “relatively short” regions of the polyprotein that comprise multiple epitopes and is preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The “relatively short” peptide is generally
 15 less than 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping
 20 (*i.e.*, frame shifted relative to one another). For example, with frame shifted overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes.

- The vaccine composition will preferably include, for example, three CTL epitopes
 25 and at least one HTL epitope from HCV. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

- 30 The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA make-up that is presently unknown.

Furthermore, this embodiment (absent analogs) directs the immune response to multiple peptide sequences that are actually present in native HCV antigens thus avoiding the need to evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions.

- 5 Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

Example 15. Polypeptidic Vaccine Compositions Directed To Multiple Diseases

- 10 The HCV peptide epitopes of the present invention are used in conjunction with peptide epitopes from target antigens related to one or more other diseases, to create a vaccine composition that is useful for the prevention or treatment of HCV as well as the one or more other disease(s). Examples of the other diseases include, but are not limited to, HIV, HBV, and HPV.

- 15 For example, a polypeptidic peptide composition comprising multiple CTL and HTL epitopes that target greater than 98% of the population may be created for administration to individuals at risk for both HCV and HIV infection. The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various disease-associated sources, or can be administered as a composition comprising
20 one or more discrete epitopes.

Example 16. Use of peptides to evaluate an immune response

- Peptides of the invention may be used to analyze an immune response for the presence of specific CTL or HTL populations directed to HCV. Such an analysis may be
25 performed in a manner as that described by Ogg *et al.*, *Science* 279:2103-2106, 1998. In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

- In this example highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") are used for a cross-sectional analysis of, for example, HCV HLA-A*0201-
30 specific CTL frequencies from HLA A*0201-positive individuals at different stages of infection or following immunization using an HCV peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al.*, *N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β 2-

- microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, $\beta 2$ -microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is
- 5 isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5'triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.
 - 10 For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 μ l of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are
 - 15 applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the HCV epitope, and thus the stage
 - 20 of infection with HCV, the status of exposure to HCV, or exposure to a vaccine that elicits a protective or therapeutic response.

Example 17: Use of Peptide Epitopes to Evaluate Recall Responses

- 25 The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be performed on patients who have recovered from infection, who are chronically infected with HCV, or who have been vaccinated with an HCV vaccine.

- For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any HCV vaccine. PBMC are
- 30 collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that are preferably highly conserved and, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 µg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 µg/ml to each well and HBV core 128-140 epitope is added at 1 µg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4×10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 µl/well of complete RPMI. On days 3 and 10, 100 ml of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously described (Rehermann, *et al.*, *Nature Med.* 2:1104,1108, 1996; Rehermann *et al.*, *J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al.*, *J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al.*, *J. Virol.* 66:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with the synthetic peptide epitope of the invention at 10 µM, and labeled with 100 µCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4-h, split well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: $100 \times [(\text{experimental release-spontaneous release})/(\text{maximum release-spontaneous release})]$. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to HCV or an HCV vaccine.

- The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of 1.5×10^5 cells/well and are stimulated
- 5 with 10 $\mu\text{g/ml}$ synthetic peptide, whole antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^3H -thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^3H -thymidine
- 10 incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^3H -thymidine incorporation in the presence of antigen divided by the ^3H -thymidine incorporation in the absence of antigen.

Example 18: Induction Of Specific CTL Response In Humans

- 15 A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study and carried out as a randomized, double-blind, placebo-controlled trial. Such a trial is designed, for example, as follows:

A total of about 27 subjects are enrolled and divided into 3 groups:

- 20 Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;
- Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;
- Group III: 3 subjects are injected with placebo and 6 subjects are injected with
- 25 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage.

- The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the
- 30 peptide composition are an index of the intrinsic activity of this the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

The vaccine is found to be both safe and efficacious.

Example 19: Phase II Trials In Patients Infected With HCV

Phase II trials are performed to study the effect of administering the CTL-HTL peptide compositions to patients having chronic HCV infection. The main objectives of the trials are to determine an effective dose and regimen for inducing CTLs in chronically infected HCV patients, to establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of chronically infected CTL patients, as manifested by a transient flare in alanine aminotransferase (ALT), normalization of ALT, and reduction in HCV DNA. Such a study is designed, for example, as follows:

The studies are performed in multiple centers. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65, include both males and females, and represent diverse ethnic backgrounds. All of them are infected with HCV for over five years and are HIV, HBV and delta hepatitis virus (HDV) negative, but have positive levels of HCV antigen.

The magnitude and incidence of ALT flares and the levels of HCV DNA in the blood are monitored to assess the effects of administering the peptide compositions. The levels of HCV DNA in the blood are an indirect indication of the progress of treatment. The vaccine composition is found to be both safe and efficacious in the treatment of chronic HCV infection.

Example 20: Alternative Method of Identifying Motif-Bearing Peptides

Another way of identifying motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for tissue typing, have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule. These cells can then be infected with a pathogenic organism, *e.g.*, HCV, HIV, *etc.* or transfected with nucleic acids that express the antigen of interest. Thereafter, peptides produced by endogenous antigen processing of peptides produced consequent to infection (or as a result of transfection) will bind to HLA molecules within the cell and be transported and displayed on the cell surface.

The peptides are then eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, *e.g.*, by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because, as disclosed herein, the majority of peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the cell.

Alternatively, cell lines that do not express any endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells may then be used as described, *i.e.*, they may be infected with a pathogenic organism or transfected with nucleic acid encoding an antigen of interest to isolate peptides corresponding to the pathogen or antigen of interest that have been presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each HLA allele expressed. Moreover, one of skill would also recognize that means other than infection or transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the

TABLE I

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	TILVMS		FWY
A2	LIVMATQ		IVMATL
A3	VSMATLI		RK
A24	YFWIVLMT		FIYWLM
B7	P		VILFMWYA
B27	RHK		FYLWMIVA
B44	ED		FWYLIMVA
B58	ATS		FWYLIVMA
B62	QLIVMP		FWYMIVLA
MOTIFS			
A1	TSM		Y
A1		DEAS	Y
A2.1	LMVQIAT		VLIMAT
A3	LMVISATFCGD		KYRHFA
A11	VTMLISAGNCDF		KRYH
A24	YFWM		FLIW
A*3101	MVTALIS		RK
A*3301	MVALFIST		RK
A*6801	AVTMSLI		RK
B*0702	P		LMFWYAIIV
B*3501	P		LMFWYIVA
B51	P		LIVFWYAM
B*5301	P		IMFWYALV
B*5401	P		ATIVLMFWY

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE II

POSITION

SUPERMOTIES

	1	2	3	4	5	6	7	8	C-terminus
A1		1° Anchor TTL/M/S							1° Anchor FWY
A2		1° Anchor LVIM/ITQ							1° Anchor LVIM/AT
A3	preferred	1° Anchor VSM/ATL	YFW (4/5)		YFW (3/5)	YFW (4/5)	P (4/5)		1° Anchor RK
	deleterious	DE (3/5); P (5/5)	DE (4/5)						
A24		1° Anchor YFW/ITLM <i>T</i>							1° Anchor FYW/ITLM
B7	preferred	FWY (5/5) LVIM (3/5)	1° Anchor P	FWY (4/5)				FWY (3/5)	1° Anchor VILF/MWYA
	deleterious	DE (3/5); P (5/5); G (4/5); A (3/5); QN (3/5)			DE (3/5)	G (4/5)	QN (4/5)	DE (4/5)	
B27		1° Anchor RHK							1° Anchor FYLM/MVLA
B44		1° Anchor E/D							1° Anchor FWYLMVA
B58		1° Anchor ATS							1° Anchor FWYLVIM/4
B62		1° Anchor QLIM/P							1° Anchor FWYMLVLA

POSITION

1	2	3	4	5	6	7	8	C-terminus
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POSITION

1	2	3	4	5	6	7	8	C-terminus
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MOTIFS

A1 9-mer	preferred	GRYW	<u>1°Anchor</u> STM	DEA	YFW	P	DEQN	YFW	<u>1°Anchor</u> Y
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deleterious	DE		<u>1°Anchor</u> RHKLI ^P VM	A	G	A			
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A1 9-mer	preferred	GRHK	ASTCLIV M	<u>1°Anchor</u> DEAS	GSTC	ASTC	LIVM	DE	<u>1°Anchor</u> Y
deleterious	A		RHKDEPY FW		DE	PQN	RHK	PG	GP

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POSITION

	1	2	3	4	5	6	7	8	9	C-terminus or C-terminus
A3	preferred	RHK	^{1°Anchor} LMVISAT FCGD	YFW	PRHKYFW	A	YFW	P	^{1°Anchor} KYRHF4	
	deleterious	DEP	DE							

A11	preferred	A	^{1°Anchor} VTLMISA GNCDP	YFW	YFW	A	YFW	YFW	P	^{1°Anchor} KRYH
	deleterious	DEP						A	G	

preferred	YFWRHK	$\frac{1^\circ \text{Anchor}}{\text{YFWM}}$	STC	YFW	YFW	$\frac{1^\circ \text{Anchor}}{\text{FLIW}}$
deleterious	DEG	DE	G	QNP	DERHK	G
						AON

A24 10-mer	preferred	^{1°Anchor} YFWM	P	YFWP	P			^{1°Anchor} FLIW
	deleterious	GDE	QN	RHK	DE	A	QN	DEA

A3101	preferred	RHK	^{1°Anchor} MTNLS	YFW	P	YFW	YFW	AP	^{1°Anchor} RK
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POSITION

	1	2	3	4	5	6	7	8	9	C-terminus
B51 preferred	LIVMF ^{1°Anchor} WY	P	FWY	STC	FWY	G	FWY	C-terminus or 1°Anchor	LIVFHY ^{1°Anchor} AM	
deleterious	AGPDERHKSTC				DE	G	DEQN	GDE		

B5301 preferred	LIVMF ^{1°Anchor} WY	P	FWY	STC	FWY	LIVMF ^{1°Anchor} WY	FWY	IMF ^{1°Anchor} WY ^{1°Anchor} ALV		
deleterious	AGPON				G	RHKQN	DE			

B5401 preferred	FWY	I°Anchor _P	FWYLI ^{1°Anchor} VM	LIVM	ALIVM	FWYAP	I°Anchor _Y			
deleterious	GPNODE	GDESTC	RHKDE	DE	QNDGE	DE				

Italicized residues indicate less preferred or "tolerated" residues.
The information in Table II is specific for D-mers unless otherwise specified.

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TABLE III

		POSITION							
MOTIFS	<div><div><div>1° anchor</div><div>1</div></div></div>	<div><div><div>2</div></div></div>	<div><div><div>3</div></div></div>	<div><div><div>4</div></div></div>	<div><div><div>5</div></div></div>	<div><div><div>1° anchor</div><div>6</div></div></div>	<div><div><div>7</div></div></div>	<div><div><div>8</div></div></div>	<div><div><div>9</div></div></div>
DR4 preferred	FMVLIW	M	T		I	VSTCPALIM	MH		MH
deletions				W			R		WDE
DR1 preferred	MFLIWHY					VMATSPIC	M		AVM
deletions		C	CH	PAMQ FD	CWD		GDE	D	
DR7 preferred	MFLIWHY	M	W	A		IVMSACTPL	M		IV
deletions		C		G			GRD	N	G
DR Supermotif	MFLIWHY					VMSTACPIL			

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DR3 MOTIFS	[1° anchor 1]	[2]	[3]	[1° anchor 4]	[5]	[1° anchor 6]
motif a preferred	LIVMFY			D		
motif b preferred	LIVMFAY			DNQEST		KRH

Italicized residues indicate less preferred or "tolerated" residues.

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Table IV. HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0205	941.01	FLPSDYFPSV	4.3
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1141.02	FTQAGYPAL	40
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2402	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLVL	5.5
B*3501	1021.05	FPFKYAAAF	7.2
B*51	1021.05	FPFKYAAAF	5.5
B*5301	1021.05	FPFKYAAAF	9.3
B*5401	1021.05	FPFKYAAAF	10

Table V. HLA Class II Standard Peptide Binding Affinity.

Allele	Nomenclature	Standard Peptide	Sequence	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2β1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2β2	553.01	QYIKANSKFIGITE	20

The "Nomenclature" column lists the allelic designations used in Tables XIX and XX.

Table VI

HLA-supertype	Allele-specific HLA-supertype members	
	Verified ^a	Predicted ^b
A1	A*01:01, A*25:01, A*26:01, A*26:02, A*32:01	A*01:02, A*26:04, A*36:01, A*43:01, A*80:01
A2	A*02:01, A*02:02, A*02:03, A*02:04, A*02:05, A*02:06, A*02:07, A*02:09, A*02:14, A*68:02, A*69:01	A*02:08, A*02:10, A*02:11, A*02:12, A*02:13
A3	A*03:01, A*11:01, A*31:01, A*33:01, A*66:01	A*03:02, A*11:02, A*26:03, A*33:02, A*33:03, A*34:01, A*34:02, A*66:01, A*66:02, A*74:01
A24	A*23:01, A*24:02, A*30:01	A*24:03, A*24:04, A*30:02, A*30:03
B7	B*07:02, B*07:03, B*07:04, B*07:05, B*15:06, B*35:01, B*35:02, B*35:03, B*35:04, B*35:05, B*35:06, B*35:07, B*35:08, B*51:01, B*51:02, B*51:03, B*51:04, B*51:05, B*53:01, B*54:01, B*55:01, B*55:02, B*56:01, B*56:02, B*67:01, B*78:01	B*15:11, B*42:01, B*59:01
B27	B*14:01, B*14:02, B*15:09, B*27:02, B*27:03, B*27:04, B*27:05, B*27:06, B*38:01, B*39:01, B*39:02, B*73:01	B*27:01, B*27:07, B*27:08, B*38:02, B*38:03, B*39:04, B*39:05, B*48:01, B*48:02, B*15:10, B*15:18, B*15:03
B44	B*18:01, B*18:02, B*37:01, B*44:02, B*44:03, B*44:04, B*40:01, B*40:02, B*40:06	B*41:01, B*45:01, B*47:01, B*49:01, B*50:01
B58	B*57:01, B*57:02, B*58:01, B*58:02, B*15:16, B*15:17	
B62	B*15:01, B*15:02, B*15:13, B*52:01	B*13:01, B*13:02, B*15:04, B*15:05, B*15:06, B*15:07, B*15:15, B*15:20, B*15:21, B*15:12, B*15:14, B*15:19

a. Verified alleles includes alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.

b. Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

HCY A01 Super Motif with Binding Information

[illegible]

Table VIII

HCY A02 Super Melt with Binding Information

Conservancy	Freq.	Position	Sequence	A' 0201	A' 0202	A' 0203	A' 0206	A' 6002
83	13	1904	MLIRRH					
86	12	1673	MLAATCL					
79	11	1250	MOGRVCL					
79	11	1250	MOGRVCL					
79	11	1250	MOGRVCL					
79	11	147	MAHALAHV					
79	11	147	MAHALAHV					
100	14	1264	MAITLFGA					
93	13	1264	MAITLFGVM					
86	12	1167	MAVCTRGV					
79	11	1187	MAVCTRGVA					
79	11	1187	MAVCTRGVAKA					
93	13	1680	ALSPGAL					
86	12	1890	ALSPGALV	0.0014				
86	12	1890	ALSPGALV	0.0035				
100	14	150	ALAHGVH					
100	14	150	ALAHGVH	0.0037				
86	12	1737	ALGLDIA					
86	12	689	ALSTGLHL					
79	11	1696	ALVGVVCA	0.0160	0.0006	0.2200	0.0002	0.0039
79	11	1896	ALVGVVCAAI					
86	12	1602	AOAPPSWDCM					
79	11	1251	AOGRVCL					
79	11	1251	AOGRVCL					
86	12	77	AOGRVCL					
93	13	1265	AITLFGVM					
79	11	1354	AITLFGVIT					
79	11	1596	ATVCAHQA					
100	14	1419	AVAYRGL	0.0002				
100	14	1419	AVAYRGL					
79	11	1188	AVCTRGVA					
79	11	1188	AVCTRGVAKA					
100	14	1917	AVCTRGVCL	0.0001				
100	14	1917	AVCTRGVCL					
93	13	1903	AVOMANILIA					
79	11	1530	CAVVELTPA	0.0002				
86	12	2341	CLMKGVPL					
86	12	709	CLMKGVPL					
79	11	1653	CHSADLEV					

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RESEARCH

IJCV A02 Super Motif with Binding Information

Consensus	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0208	A*5802
86	12	1870	GVLMALIA					
86	12	1870	GVLMALIAITL					
79	11	181	GVNITITL	0.0001				
68	12	43	GVNITIT					
100	14	2819	GVNCEMA					
100	14	2819	GVNCEMA	0.0002				
100	14	2818	GVNCEML	0.0001				
92	13	154	GVNCEAV					
79	11	1900	GVNCDL					
100	14	1234	GVNCSGST					
100	14	1312	GVNFFISIT					
66	12	636	GVNIMV	0.0100	0.0014	0.5400	0.0027	0.0037
79	11	1719	GVNIEGSA					
79	11	1719	GVNIEGSA					
78	11	2855	GVNIEGSA	0.3300	0.0004	0.1300	0.0280	0.0053
78	11	2855	GVNIEGSA					
86	12	2855	GVNIEGSA					
86	12	2855	GVNIEGSA					
79	11	1910	GVNIEGSA					
79	11	1910	GVNIEGSA					
86	12	1923	GVNIEGSA					
86	12	1923	GVNIEGSA					
100	14	1925	GVNIEGSA					
79	11	1856	GVNIEGSA	0.0430	0.0300	2.0000	0.0049	0.0450
79	11	1856	GVNIEGSA	0.0002				
86	12	1816	GVNIEGSA					
86	12	1816	GVNIEGSA	0.0430	0.0024	0.0190	0.0005	0.0038
86	12	1816	GVNIEGSA					
86	12	1331	GVNIEGSA					
86	12	1331	GVNIEGSA					
93	13	1891	GVNIEGSA	0.0210	0.0004	0.3700	0.0036	0.0130
93	13	1891	GVNIEGSA					
93	13	1891	GVNIEGSA					
79	11	2591	GVNIEGSA	0.0088				
100	14	1777	GVNIEGSA					
100	14	1777	GVNIEGSA					
100	14	1777	GVNIEGSA					
86	12	2250	GVNIEGSA					
86	12	2250	GVNIEGSA					
100	14	2816	GVNIEGSA					
100	14	2816	GVNIEGSA					
100	14	2816	GVNIEGSA					
86	12	989	GVNIEGSA					
86	12	989	GVNIEGSA					

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HCV A02 Super Molt with Binding Information

Consistency	Freq	Position	Sequence	A '0201	A '0202	A '0203	A '0206	A '0208
79	11	1296	INTSYGSHL					
79	11	1296	INTSYGSHL					
79	11	2613	IMFDLGV					
79	11	2613	IMFDLGV	0.0018				
93	13	30	MGSVYL					
86	12	1730	KALGSLDT					
86	12	1730	KALGSLDT					
86	12	2625	KMALYDVL					
86	12	1734	KOKAGSL					
86	12	1734	KOKAGSLDT					
86	12	1734	KOKAGSLDT					
86	12	121	KMDITGFA					
86	14	1255	KVLNPSV	0.0048				
100	14	1255	KVLNPSV					
100	14	1255	KVLNPSV					
100	14	1255	KVLNPSV					
79	11	1244	KVPAAYAA					
79	11	1244	KVPAAYAA					
79	12	1872	LALANVCL	0.0011				
86	11	1305	LADGSGGA					
86	12	1729	LAEGRCKA					
86	12	1729	LAEGRCKA					
79	11	1057	LAEGRGAY					
79	11	1057	LAEGRGAY					
79	11	1057	LAEGRGAY					
79	11	1057	LAEGRGAY					
100	14	151	LAEGRNVL					
86	12	179	LALSLT					
86	12	972	LAVNEPV					
79	11	1924	LMEASGRNV					
100	14	2815	LUTSGSNV	0.0004				
100	14	2815	LUTSGSNV					
100	14	2812	LUTSGSNV					
79	11	2812	LUTSGSNV	0.0002				
79	11	2812	LUTSGSNV					
86	12	178	LUALSCL					
86	12	178	LUALSCL					
100	14	726	LULFLDAA	0.0230	0.0150	0.0220	0.0011	0.0130
93	13	726	LULFLDAA					
86	12	1812	LULFLDAA	1.2000	0.0380	3.1000	0.1800	1.2000
93	13	729	LULADAV					
93	13	1887	LUPALISGAL	0.0081				
93	13	1887	LUPALISGAL					
93	13	36	LUPHGPFL	0.0025				
93	13	36	LUPHGPFL					

	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440	2441	2442	2443	2444	2445	2446	2447	2448	2449	2450	2451	2452	2453	2454	2455	2456	2457	2458	2459	2460	2461	2462	2463	2464	2465	2466	2467	2468	2469	2470	2471	2472	2473	2474	2475	2476	2477	2478	2479	2480	2481	2482	2483	2484	2485	2486	2487	2488	2489	2490	2491	2492	2493	2494	2495	2496	2497	2498	2499	2500	2501	2502	2503	2504	2505	2506	2507	2508	2509	2510	2511	2512	2513	2514	2515	2516	2517	2518	2519	2520	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530	2531	2532	2533	2534	2535	2536	2537	2538	2539	2540	2541	2542	2543	2544	2545	2546	2547	2548	2549	2550	2551	2552	2553	2554	2555	2556	2557	2558	2559	2560	2561	2562	2563	2564	2565	2566	2567	2568	2569	2570	2571	2572	2573	2574	2575	2576	2577	2578	2579	2580	2581	2582	2583	2584	2585	2586	2587	2588	2589	2590	2591	2592	2593	2594	2595	2596	2597	2598	2599	2600	2601	2602	2603	2604	2605	2606	2607	2608	2609	2610	2611	2612	2613	2614	2615	2616	2617	2618	2619	2620	2621	2622	2623	2624	2625	2626	2627	2628	2629	2630	2631	2632	2633	2634	2635	2636	2637	2638	2639	2640	2641	2642	2643	2644	2645	2646	2647	2648	2649	2650	2651	2652	2653	2654	2655	2656	2657	2658	2659	2660	2661	2662	2663	2664	2665	2666	2667	2668	2669	2670	2671	2672	2673	2674	2675	2676	2677	2678	2679	2680	2681	2682	2683	2684	2685	2686	2687	2688	2689	2690	2691	2692	2693	2694	2695	2696	2697	2698	2699	2700	2701	2702	2703	2704	2705	2706	2707	2708	2709	2710	2711	2712	2713	2714	2715	2716	2717	2718	2719	2720	2721	2722	2723	2724	2725	2726	2727	2728	2729	2730	2731	2732	2733	2734	2735	2736	2737	2738	2739	2740	2741	2742	2743	2744	2745	2746	2747	2748	2749	2750	2751	2752	2753	2754	2755	2756	2757	2758	2759	2760	2761	2762	2763	2764	2765	2766	2767	2768	2769	2770	2771	2772	2773	2774	2775	2776	2777	2778	2779	2780	2781	2782	2783	2784	2785	2786	2787	2788	2789	2790	2791	2792	2793	2794	2795	2796	2797	2798	2799	2800	2801	2802	2803	2804	2805	2806	2807	2808	2809	2810	2811	2812	2813	2814	2815	2816	2817	2818	2819	2820	2821	2822	2823	2824	2825	2826	2827	2828	2829	2830	2831	2832	2833	2834	2835	2836	2837	2838	2839	2840	2841	2842	2843	2844	2845	2846	2847	2848	2849	2850	2851	2852	2853	2854	2855	2856	2857	2858	2859	2860	2861	2862	2863	2864	2865	2866	2867	2868	2869	2870	2871	2872	2873	2874	2875	2876	2877	2878	2879	2880	2881	2882	2883	2884	2885	2886	2887	2888	2889	2890	2891	2892	2893	2894	2895	2896	2897	2898	2899	2900	2901	2902	2903	2904	2905	2906	2907	2908	2909	2910	2911	2912	2913	2914	2915	2916	2917	2918	2919	2920	2921	2922	2923	2924	2925	2926	2927	2928	2929	2930	2931	2932	2933	2934	2935	2936	2937	2938	2939	2940	2941	2942	2943	2944	2945	2946	2947	2948	2949	2950	2951	2952	2953	2954	2955	2956	2957	2958	2959	2960	2961	2962	2963	2964	2965	2966	2967	2968	2969	2970	2971	2972	2973	2974	2975	2976	2977	2978	2979	2980	2981	2982	2983	2984	2985	2986	2987	2988	2989	2990	2991	2992	2993	2994	2995	2996	2997	2998	2999	3000
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ILCV A02 Super Motif with Binding Information

Conservancy	Freq.	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*0802
79	11	2916	RLHKAESL					
79	11	2611	RLHFPOL	0.0280	0.0055	0.0180	0.0002	0.0032
79	11	2611	RLHFPOLCV					
79	11	1816	RLHPTLHGT	0.0890	0.0110	1.0000	0.0100	0.0050
86	12	1029	RLHAPTA					
86	12	1347	RLWLATA					
86	12	1347	RLVLATAT					
100	14	619	RLHNPCT					
86	12	317	RLHWDMM					
93	13	635	RLHVGSEHFL					
88	12	2243	RLHAKZN					
88	12	2243	RLHAKSNT					
88	12	2243	RLHAKSNTW					
79	11	1284	RLHGNHTT					
79	11	1284	RLHGNHTT					
100	14	2621	RLHCEMAL					
86	12	2621	RLHCEMALYV					
86	12	2552	RLHESKNV					
86	12	2552	RLHESKNV					
79	11	2100	RLHGRHTV	0.0001				
86	12	158	RLHDSQVAT					
86	12	158	RLHDSQVAT					
86	12	2833	RLHPTLHPT					
86	11	1555	RLHSDVPT					
78	11	1555	RLHSDVPT					
78	11	2212	RLHSPKAT					
78	11	2212	RLHSPKAT					
93	13	2207	RLHSPKAT					
100	14	175	RLHLLAL					
88	12	175	RLHLLALSL					
100	14	1470	RLHPTTL					
86	12	1470	RLHPTTLT					
79	11	1470	RLHPTTLT					
79	11	2826	RLHSPRGE					
86	12	1051	RLHSPRGE	0.0008				
100	14	2178	RLHSPRSH	0.0002				
100	14	2178	RLHSPRSH	0.0053				
100	14	2178	RLHSPRSH					
86	12	2163	RLHSPRSHV					
83	13	2209	RLHSPSLA					
79	11	2209	RLHSPSLA					
79	11	2209	RLHSPSLA					

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

LCV A02 Super Motif with Binding Information

Consensus	Freq	Position	Sequence	A*0201	A*0202	A*0203	A*0208	A*5802
93	13	56	SCAPRRQDA					
86	12	1242	STKVPAYVA					
79	11	1242	STKVPAYVA					
100	14	1784	STLPGNPA					
79	11	1784	STLPGNPA	0.0007				
79	11	2	STNPPRRQKT					
88	12	1663	STNVLVGSV					
86	12	1663	STNVLVGSVL					
86	12	1663	STNVLVGSVLA					
86	12	1299	STVGKFLA					
100	14	1282	SVANLIFGCA					
86	12	1455	SVNDKNCV	0.0088				
86	12	1455	SVNDKNCVYT					
86	12	995	TACGDH					
86	12	1343	TAGARLVV					
86	12	1343	TAGARLVVLA					
88	12	1343	TAGARLVVLA					
79	11	1343	TAGARLVVLA					
79	11	2852	TARHPNSVWL					
79	11	2590	TIAMKNEV					
93	13	1288	TIARFANVA					
88	12	1288	TIARFANVSKA					
79	11	1622	TIHSPFL					
79	11	1622	TIHSPFL	0.0070				
96	12	1811	TIHNSGV					
79	11	1811	TIHNSGV	0.0003				
79	11	1811	TIHNSGV	0.0004				
79	11	1745	TIHNSGV					
88	12	125	TIHNSGV					
88	12	125	TIHNSGV	0.0003				
79	11	2871	TIHNSGV					
79	11	2871	TIHNSGV					
79	11	2871	TIHNSGV					
86	12	1208	TIHNSGV					
86	12	1208	TIHNSGV					
86	12	1484	TIHNSGV					
79	11	2589	TIHNSGV					
79	11	685	TIHNSGV					
79	11	685	TIHNSGV					
79	11	1208	TIHNSGV					
66	12	1208	TIHNSGV					
79	11	2739	TIHNSGV					

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ICCV A02 Super Motif with Binding Information

Consistency	Frq.	Position	Sequence	A'0201	A'0202	A'0203	A'0206	A'6802
100	14	1918	VOMNNTLU					
100	14	1918	VOMNNTLU					
100	14	1916	VOMNNTLUFA					
86	12	1463	VOTOTDESL					
79	11	1136	VTRHADIV					
79	11	1136	VTRHADIV					
86	12	1661	VTSIWWLV					
86	12	1661	VTSIWWLVGSV					
79	11	1439	VVATDALM					
79	11	1439	VVATDALMT					
79	11	1901	VVCALDINH					
79	11	1898	VVEVCAIA					
79	11	1898	VVEVCAIAI					
86	12	1660	VVSTIWWL					
86	12	1660	VVSTIWWL					
86	12	1766	VWAKIMNFI	0.0003				
86	12	1766	VWAKIMNFI	0.0001				
86	12	2673	VWDPGTWFL					
79	11	2287	VWHRMLMT					
100	14	1920	VWHRDNPFL					
79	11	557	VWNSIGET	0.0410	0.0330	3.0000	0.0023	0.1000
86	12	1665	VWVSGNLL					
86	12	1665	VWVSGNLL	0.0005				
86	12	1665	VWVSGNLL	0.0015				
79	11	1248	VYADSNVFL					
79	11	1248	VYADSNVFL					
79	11	1248	VYADSNVFL					
79	11	1248	VYADSNVFL					
79	11	136	VYRVSHPFL	0.0050				
100	14	1779	VYSGSITL					
86	12	1165	VYSGSISGFL	0.0002				
86	12	1165	VYSGSISGFL					
93	13	35	VYTHPCFL	0.0400				
79	11	2836	VYTRPPT	0.0007	0.0220	0.0089		0.0039
86	12	1590	VYVAVOAT					
86	12	1590	VYVAVOATV	0.2500	0.1100	0.5300	0.0450	1.2000
86	12	1590	VYVAVOATVCA					
86	12	1138	VYVTRIDVFL	0.0110	0.0021	2.8000	0.0520	0.0130
79	11	1136	VYVTRIDVFL					
93	13	1594	VYVATVCAIA					

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HCV AD2 Sweet Motif with Binding Information

Consensus	From	Position	Sequence	A*0201	A*0202	A*0203	A*0206	A*8902
79	11	1584	YQATVCAHQDA					
79	11	1106	YINMOOL					
79	11	1106	YINMOOLV					
86	12	276	YVGLGCSV	0.0018				
86	12	276	YVGLGCSFL					
93	13	637	YVGSVEHL	0.0008				
86	12	1939	YVPESDMA					
86	12	1939	YVPESDMAA					
86	12	1939	YVPESDMAHV					
86	12	1939	555					

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HCY Δ93 Super Motif (With Binding Information)

[illegible]

HCY ADJ Sinter Mod(Cylin Binding Information)

Conservancy	Freq	Position	Sequence	A*0301	A*1101	A*1301	A*1301	A*8801
86	12	2235	LEAKLW	0.0008	0.0005	0.0018	0.0089	0.0008
100	14	1386	LFOISKK	0.5400	0.1800	0.0071	0.0012	0.0240
100	14	2812	LWFOUGN	0.0003	0.0001			
79	14	726	LELLADAR					
100	13	36	LPRNEPR					
68	12	97	LSPHESR					
79	11	1591	LWADATVCAH					
79	11	1	MSINPQCA					
79	11	1	MSINPQCA					
86	12	2249	NITWESRK	0.0010	0.0062			
79	11	14	NINPRQCK	0.0010	0.0007			
79	11	1285	PITSTGK					
79	11	2667	PMKSYDIA					
93	13	514	PSWVAGITIA					
79	11	1607	PSWQAMK					
86	12	109	PTDPTSR	0.0008	0.0005	0.0008	0.0008	0.0002
83	13	1528	PTDPTSR	0.0002	0.0001			
93	13	518	PWQVITIA	0.0008	0.0005			
93	13	1340	QMETIAR					
93	13	29	QWGGVILPR					
68	12	289	QUTFSPI					
79	11	289	QUTFSPI	0.7500	0.0330	0.0280	0.0077	3.1000
79	11	2210	QLSAPSLK					
79	11	1166	PAWCTGIVAK					
100	14	149	PAIAGVR					
79	11	47	PAITKTSR					
79	11	43	PLGVAMR	0.8400	0.0290	0.0420	0.0004	0.0001
70	11	1823	PLHAPSR					
100	14	2811	PLWPGVIA					
79	11	636	PLWPGVIA	0.7200	0.0200	0.1800	0.0030	0.0045
100	13	45	PSKQVIA					
93	13	2207	SACKLAPSLK					
79	11	1132	SERXVIA	0.0003	0.0044			
96	12	1	SINPQCA					
79	11	2	SINPQCA					
79	11	2	SINPQCA					
79	11	2	SINPQCA					
66	12	1266	TLGQVANSK	0.0810	0.0610	0.0005	0.0013	0.0009
79	11	1622	TLHGPIILYR					
93	13	52	TSEESQRI					
86	12	52	TSEESQRI	0.0003	0.0001			
86	12	1050	TSEESQRI					
86	12	1884	TSATIRK	0.2400	0.8900	0.0048	0.0025	0.0310
79	11	1592	VAGQVIA	0.0005	0.0036	0.0680	0.0720	0.0280
86	12	1337	VADQETVCA					
79	11	1338	VIRHAPVIR					
79	11	1338	VIRHAPVIR					
79	11	1801	VICQALIR					
79	11	1886	VICQALIR					
93	13	517	WQGITIA					

HCY A01 Super Motif (With Binding Information)

Consensus	Freq	Position	Sequence	A*0301	A*1101	A*3101	A*3301	A*5601
80	12	93	WAKLSPH					
80	12	93	WALFESH	0.0008	0.0005			
100	14	1890	WAKLSPH					
100	11	557	WAKLSPH	0.0530	0.0010	0.0014		
79	11	35	WAKLSPH	0.0054	0.0005		0.0420	0.0056
73	13	2930	WAKLSPH					
71	14	637	WAKLSPH					
100	12	1939	WAKLSPH	0.0003	0.0001			
80		112	WAKLSPH					

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Table X

HCY A24 Super Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A-2401
ALLSPQAL	1890	8	13	93	
ALHAEVYML	150	9	12	100	
ALSTGLHL	689	8	12	86	
ALVGVGVGAKAI	11	11	11	78	
ATCNLFQCSF	1896	10	13	93	
ATLGFQAY	165	10	12	100	
ATLGFQAYM	1265	6	13	100	
AVVYRGL	1419	8	14	100	
AVQWMAERL	1917	8	14	100	
AVQWMAERLI	1817	9	14	100	
AVQWMAERLI	1917	11	14	100	
AVQWMAERLI	1817	8	12	86	
AVQWMAERLI	1917	8	11	79	0.0009
AVQWMAERLI	1817	10	11	100	
AVQWMAERLI	1917	11	14	100	
AVQWMAERLI	1817	10	12	86	
AVQWMAERLI	1917	10	12	86	
AVQWMAERLI	1817	8	11	79	0.0001
AVQWMAERLI	1917	9	11	79	
AVQWMAERLI	1817	10	11	79	
AVQWMAERLI	1917	11	11	79	
AVQWMAERLI	1817	9	11	79	
AVQWMAERLI	1917	10	12	86	
AVQWMAERLI	1817	8	12	86	
AVQWMAERLI	1917	8	12	86	
AVQWMAERLI	1817	10	12	86	
AVQWMAERLI	1917	8	11	79	
AVQWMAERLI	1817	9	11	79	
AVQWMAERLI	1917	10	14	100	
AVQWMAERLI	1817	8	14	100	
AVQWMAERLI	1917	10	14	100	
AVQWMAERLI	1817	8	12	86	
AVQWMAERLI	1917	9	12	86	
AVQWMAERLI	1817	11	12	86	
AVQWMAERLI	1917	10	13	93	
AVQWMAERLI	1817	8	11	79	
AVQWMAERLI	1917	9	11	79	
AVQWMAERLI	1817	10	12	86	
AVQWMAERLI	1917	8	12	86	
AVQWMAERLI	1817	10	12	86	
AVQWMAERLI	1917	8	11	79	
AVQWMAERLI	1817	9	11	79	
AVQWMAERLI	1917	10	14	100	
AVQWMAERLI	1817	8	14	100	
AVQWMAERLI	1917	10	14	100	
AVQWMAERLI	1817	8	12	86	
AVQWMAERLI	1917	9	12	86	
AVQWMAERLI	1817	11	12	86	
AVQWMAERLI	1917	10	13	93	
AVQWMAERLI	1817	8	11	79	
AVQWMAERLI	1917	9	11	79	
AVQWMAERLI	1817	10	12	86	
AVQWMAERLI	1917	8	12	86	
AVQWMAERLI	1817	10	14	100	
AVQWMAERLI	1917	8	14	100	
AVQWMAERLI	1817	10	14	100	
AVQWMAERLI	1917	8	12	86	
AVQWMAERLI	1817	9	12	86	
AVQWMAERLI	1917	11	12	86	
AVQWMAERLI	1817	10	13	93	
AVQWMAERLI	1917	8	11	79	
AVQWMAERLI	1817	9	11	79	
AVQWMAERLI	1917	10	12	86	
AVQWMAERLI	1817	8	12	86	
AVQWMAERLI	1917	9	12	86	
AVQWMAERLI	1817	11	12	86	
AVQWMAERLI	1917	10	13	93	
AVQWMAERLI	1817	8	11	79	
AVQWMAERLI	1917	9	11	79	
AVQWMAERLI	1817	10	12	86	
AVQWMAERLI	1917	8	12	86	
AVQWMAERLI	1817	10	14	100	
AVQWMAERLI	1917	8	14	100	
AVQWMAERLI	1817	10	14	100	
AVQWMAERLI	1917	8	12	86	
AVQWMAERLI	1817	9	12	86	
AVQWMAERLI	1917	11	12	86	
AVQWMAERLI	1817	10	13	93	
AVQWMAERLI	1917	8	11	79	
AVQWMAERLI	1817	9	11	79	
AVQWMAERLI	1917	10	12	86	</

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

Sequence

[illegible]

ICV A24 Super Motif With Binding Information

[illegible][illegible]

ILCV A24 Super Motif With Binding Information

Sequence	Position	Pepptide No.	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
RLAPITAY	1029		9	12	86	
RLAWDMAM	317		8	12	86	
RLAWDMAMW	2875		10	12	86	
RLALMTIF	2875		8	12	86	
RLALMTIFF	2875		9	12	86	
RLVGVGBRL	835		11	13	83	
RLVCEMAL	2621		6	14	100	
RLVCEMALY	2621		9	14	100	
RLVCEMALY	156		9	14	100	
RLVCEMALY	173		9	12	86	
RSFRLTAL	173		10	14	100	
RSFRLTALL	175		8	14	100	
SRLLALSL	175		11	14	100	0.0041
SLDPTFI	1470		11	14	100	
SLDPTFI	2928		8	12	86	
SLDPTFI	2928		10	14	100	
SLDPTFI	2178		9	11	79	
SLDPTFI	1242		8	12	100	
SLDPTFI	1784		9	11	86	
SLDPTFI	1683		10	12	79	
SLDPTFI	1282		8	14	86	
SLDPTFI	1282		11	14	100	
SLDPTFI	1683		9	14	100	
SLDPTFI	2860		8	11	79	
SLDPTFI	1164		11	12	86	
SLDPTFI	2590		11	12	86	
SLDPTFI	1266		8	11	79	
SLDPTFI	1622		9	13	93	
SLDPTFI	1622		8	11	78	
SLDPTFI	1622		10	11	79	
SLDPTFI	1811		10	11	79	
SLDPTFI	1811		9	12	80	
SLDPTFI	686		11	11	79	
SLDPTFI	686		10	11	79	
SLDPTFI	1785		8	11	79	
SLDPTFI	125		9	12	86	
SLDPTFI	125		10	12	86	
SLDPTFI	2871		8	11	86	
SLDPTFI	2871		9	11	79	
SLDPTFI	2589		10	11	79	
SLDPTFI	685		11	11	79	
SLDPTFI	1208		11	11	79	
SLDPTFI	2739		8	12	86	
SLDPTFI	1486		10	12	79	
SLDPTFI	556		9	11	86	
SLDPTFI	1664		9	12	86	

0.0001

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ILCV A24 Super Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*2401
TSTVQCF	1287	8	13	93	
TSTVQDL	1287	9	12	88	
WTGTHH	1566	8	13	92	0.0230
WDLTQCF	1722	9	13	92	
VLALAAAY	1671	8	12	86	
VLALAAVCL	1671	10	12	86	
WLRQNY	1597	8	12	86	0.0070
VLPNSVAIL	1286	10	14	100	
VLTSCQNTL	2277	10	11	79	
VAVDLAGY	1842	9	11	79	
VLVGVVAIL	1842	10	11	79	
WAGSTGYF	2030	10	12	86	
WAGSTGYF	2030	0	11	79	
VTCVQVSL	1463	10	11	79	
VTRVADN	1138	9	12	86	
VWADALM	1439	8	11	79	
VWGVCCAL	1898	8	11	79	
VWGVCCAL	1898	10	11	79	
VTRVIVNL	1660	8	12	79	
VTRVTRPFL	1660	11	13	86	
WVAFLEAPL	34	8	14	100	0.0016
WVAFLEAPL	1920	8	12	86	
WVAFLEAPL	1665	11	12	86	
WVAFLEAPL	1665	11	12	86	
WVAFLEAPL	1665	9	11	79	
VLALSTL	1729	8	14	100	
VLAGSSQAPL	1165	10	12	86	
VLPTRPNTL	35	10	12	86	
VTVTHADVI	1136	10	13	93	0.0001
VTVNCOX	1106	0	11	79	
VTVNCOX	1106	11	11	79	
VTVNCOX	1106	11	11	79	
VWGLQCSNLF	276	11	12	86	
VWGLQCSNLF	276	9	12	86	
WVGVNFI	637	13	13	93	
YTRQLDLSVI	1422	10	14	100	
260		3			

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HCY B07 Super Motif (with Binding Information)

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HCY B07 Super Motif (with Binding Information)

Conservancy	Freq	Position	Sequence	B*0.02	B*3.501	B*5.101	B*5.301	B*5.401
88	12	78	CGGYYWLY	0.0001	0.0011	0.0002	0.0001	0.0002
93	13	57	CPGYYHFL	0.2300	0.0002		0.0001	0.0002
79	11	2289	PPQYHFL	0.0050				
93	13	1893	SPQLVGVV	0.0001	0.0002	0.0002	0.1200	0.0002
79	11	1893	SPQLVGVV	0.0130	0.0001	0.0018	0.0001	0.0003
79	11	2831	SPGNNV	0.0007				
79	11	2831	SPGNNV	0.0003	0.0001		0.0002	0.0037
79	11	2649	SPGNNV	0.0027				
79	11	2649	SPGNNV	0.1200	0.0002	0.0002	0.0001	0.0002
79	11	89	SPGNNV	0.3800	0.0002	0.0005	0.0001	0.0002
86	12	1935	SPGNNV	0.0001				
86	12	1975	SPGNNV	0.0026				
86	12	1975	SPGNNV	0.0001	0.0001			
79	11	1126	SPGNNV	0.0001	0.0001	0.0002	0.0001	0.0003
79	11	1126	SPGNNV	0.0001				
86	12	223	SPGNNV	0.0001				
86	12	223	SPGNNV	0.0001				
93	13	1550	SPGNNV	0.0001	0.0001	0.0001	0.0002	0.2300
93	13	1627	SPGNNV	0.0003				0.0110
83	13	1627	SPGNNV	0.0120	0.0001	0.0008	0.0001	0.0003
88	12	2858	SPGNNV	0.0001	0.0001	0.0053	0.0008	
88	12	2858	SPGNNV	0.0001				
88	12	1940	SPGNNV	0.0022				
88	12	1940	SPGNNV	0.0001	0.0001			
88	12	799	SPGNNV	0.0021				
88	12	799	SPGNNV	0.0001	0.0001	0.0010	0.0001	0.0003
100	14	616	SPGNNV	0.0001				

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HCV B27 Super Motif

Sequence	Position	No of Amino Acids	Sequence Frequency	Conservancy (%)
ARKHNNN	1767	8	12	86
AKNEFCY	2593	8	12	86
ARALAHGV	148	8	14	86
DSELSPL	663	8	11	79
EKGGRPA	2603	8	11	79
EKMALYDV	2624	8	12	86
FKQALQ	1733	8	12	86
GHRMAMOM	315	8	13	93
GKSTKPA	1240	8	12	86
GHPAPU	2806	8	11	79
HNMAMDM	316	8	13	93
KGEHL	1330	8	11	79
KGTHL	1283	8	11	79
KKQDELA	1403	8	14	100
KKQDELA	1402	8	14	100
KKQDELA	1823	8	11	79
LHONIV	887	8	12	86
LPOALNV	583	8	12	86
LNSTRTY	1832	8	11	79
NRGROR	58	8	12	86
PROSPRV	100	8	12	86
PRGRSL	112	8	11	79
RHADVIV	1140	8	12	86
RITPNSV	2054	8	12	86
PKLQPL	2843	8	12	86
RKPAPU	2607	8	11	79
RHCYAGV	2730	8	13	93
RFPAGV	38	8	13	93
RPPDVF	17	8	12	86
SKQDEL	1401	8	14	100
SKQDEL	118	8	12	86
TKLHVI	1571	8	13	93
TKPAPVA	2985	8	12	86
TICOSTY	1243	8	14	100
TRQAKAV	2874	8	14	100
VWCEKMA	1110	8	11	79
VWCEKMA	2620	8	14	100
VWCEKMA	155	8	14	100
VWCEKMA	1423	8	14	100
VWCEKMA	155	8	14	100
YRGLDSV	1423	8	14	100
YRGLDSV	2853	8	11	79
YRGLDSV	2810	8	11	79
YRGLDSV	2810	8	11	79
YRGLDSV	2874	8	12	86
YRGLDSV	2298	8	11	79
YRGLDSV	663	8	11	79

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HCV B27 Super Motif

[illegible]

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	

HCV H2Z Super Multi

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
YKALVLPSTVA 136	1254	11	14	100

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HCV B58 Super Motif

Table XIII

Sequence	Position	No. of Amino Acids	Sequence Frequency	Consistency (%)
ALURRY	1804	8	13	83
ALAAYCL	1673	8	12	86
MOGYKYL	1250	8	11	79
ALIGFGA	1264	8	14	100
AVCTRGV	1187	8	12	88
ASLWAFI	1793	8	11	78
ASSASQL	2204	8	14	100
ALIGFAY	1285	8	14	100
CSFRIPL	172	8	12	86
CSGQADI	1310	8	14	100
CSNSIVA	2819	8	11	78
CTGSSSL	1128	8	11	79
CTGCVKA	1180	8	12	86
DTAACGI	1244	8	12	86
DTICGPA	1244	8	11	79
EMLEKAV	2734	8	100	
EMLEKAV	2734	8	12	86
EMLEKAV	1942	8	12	86
EMLEKAV	1342	8	12	86
ETMOARV	1207	8	13	93
ETMNSPV	130	8	14	100
FALMGVI	1927	8	14	100
FASGNAV	174	8	11	78
FSRIPL	2870	8	14	100
FSYDRCF	2792	8	11	78
FTEAMTY	512	8	13	93
FTSPWV	1861	8	12	86
GAGVAGL	1253	8	12	86
GAHWGLA	1861	8	11	78
GALVGV	1106	8	12	86
GALVGLA	1238	8	13	86
GSASINV	1131	8	12	86
GSGLTV	1108	8	11	79
GSGLTV	2841	8	11	79
GSGLTV	2083	8	11	79
GSGLTV	2928	8	12	86
GSGLTV	2955	8	14	100
GSGLTV	1774	8	14	100
GSGLTV	2816	8	12	86
GSGLTV	989	8	12	86
GSGLTV	1241	8	11	78
GSGLTV	1857	8	14	100
GSGLTV	151	8	11	78
GSGLTV	151	8	11	78
GSGLTV	2211	8	11	78

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HCV B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy [%]
LSPGALVY	1882	8	13	93
LSTGALH	690	8	12	86
LTCGFADL	126	8	12	86
LTHDAHF	1570	8	13	93
MSAKLEEV	1654	8	11	79
NSMIGNI	2959	8	14	100
NTCYDTV	1460	8	12	88
NINSGWH	416	8	13	93
PALSPGA	1889	8	13	93
PALSTGI	688	8	12	86
PILVWANI	2070	8	11	79
PITLVYRL	1828	8	14	100
QATGARA	1895	8	13	93
QATGARA	3839	8	14	100
RSRSEPL	844	8	11	86
RSRNLGV	115	8	12	86
SAFSLSY	2923	8	11	78
SSASQLSA	2206	8	14	100
STNVPAY	1242	8	12	86
STLFGNPA	1784	8	14	100
STLPLQMA	2633	8	12	86
STVGREA	1289	8	12	86
TACQEDA	935	8	12	86
TACQEDV	1343	8	12	86
TITRSENF	1343	8	12	86
TITSCNTI	2728	8	11	78
VAGALVAF	1864	8	12	86
VTRHADVI	1136	8	11	79
VSTWLVV	1661	8	12	86
WAKLWNNF	1766	8	14	100
WAKVLNM	388	8	12	86
WAKFGFNV	76	8	12	86
YAAQGVV	1249	8	11	79
YSEHDL	2905	8	11	79
YSTVGRL	1298	8	12	86
YTNQDCL	1106	8	11	79
YTNQDCL	2758	8	16	114
YTNQDCL	1280	8	11	78
YTNQDCL	1447	8	11	78
YATLGKAY	1264	8	11	100
YAVICRGVA	1187	8	13	78
YASQLSPAS	2208	8	13	93
YATLFGANVA	1265	8	26	186
YATVCAHQA	1598	8	11	79
YCALIRRVV	1903	8	13	93

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HCV H58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Consensus [K]
CWVELTFA	1530	9	11	79
CSERFLA	172	9	14	100
CSGGADLI	1310	9	12	86
CTCCSSLY	1128	9	11	79
CTREYAKAV	1180	9	11	79
CTWANSIGF	555	9	11	79
DACQWYEL	1527	9	12	86
DTACGDIH	394	9	13	93
DTRCHSTV	2873	9	12	86
ETAGRIWV	1202	9	12	86
ETWNSVVF	1774	9	14	100
FSRIILAL	1469	9	14	100
FSRIIPFI	1567	9	13	90
FSRIISLV	1861	9	12	86
GVGVAGLY	1856	9	12	100
GVVVRKIM	1856	9	14	100
GVVVRKIM	1816	9	14	100
GVVVRKIM	1400	9	11	79
GVVVRKIM	1400	9	12	86
GVVVRKIM	222	9	12	86
GVVVRKIM	989	9	12	86
GVVVRKIM	1236	9	12	86
GVVVRKIM	1736	9	12	86
GVVVRKIM	1241	9	12	86
GVVVRKIM	1672	9	12	86
GVVVRKIM	1722	9	12	86
GVVVRKIM	356	9	14	100
GVVVRKIM	1857	9	11	79
GVVVRKIM	2822	9	11	79
GVVVRKIM	1789	9	11	79
GVVVRKIM	126	9	24	100
GVVVRKIM	2180	9	14	100
GVVVRKIM	1052	9	13	86
GVVVRKIM	1570	9	11	79
GVVVRKIM	2738	9	12	86
GVVVRKIM	2592	9	12	86
GVVVRKIM	318	9	13	93
GVVVRKIM	1418	9	13	93
GVVVRKIM	2481	9	24	100
GVVVRKIM	2658	9	12	86
GVVVRKIM	14	9	13	93
GVVVRKIM	1889	9	14	100
GVVVRKIM	1281	9	14	100
GVVVRKIM	1451	9	11	79
GVVVRKIM	2870	9	11	79

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HCY B58 Super Motif

[illegible]

THE **WORLD'S** **LARGEST**

Figure 1 shows the step-by-step construction of a minimum spanning tree. The process starts with a graph of 6 nodes and 10 edges. Edges are selected in order of increasing weight, skipping those that would either create a cycle or result in a vertex with a degree greater than 2. The final tree consists of 5 edges with a total weight of 5.

HCY_B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
WATIGRAY	1263	10	14	100
VTRGPRSGM	1507	10	16	114
VTRIAQVRY	1138	10	11	79
WAGQTPVRL	76	10	12	86
WAKHLATHF	2873	10	12	86
WAPQVYPL	2297	10	11	79
WAGQYVRL	1249	10	11	79
YRSGENRL	2930	10	11	79
YRSGENRL	2548	10	11	79
AKQALAEVRL	147	11	11	89
AKSLRTGRL	2786	11	12	89
AKSLRTGRL	1139	11	12	89
ASLPIREGRL	1717	11	14	100
ASQLSAPRLA	2208	11	11	79
CAMQAPPSW	1599	11	11	79
CSFRLTLL	172	11	14	100
CTGSSSLALV	1128	11	11	79
CTGQVAKAPF	1150	11	11	79
DTGQVAKAPF	732	11	16	114
DTTGCGRLM	1421	11	17	117
ETNGARLVLA	1342	11	12	86
FQLMKGRVL	130	11	11	79
FLLSYSGER	2925	11	11	79
FQLTHDAF	1567	11	13	93
FTLLPALSTGL	684	11	11	79
GADTMAQGLF	992	11	12	86
GAGVACGLAF	1861	11	12	86
GALVAVYDCA	1885	11	12	86
GAGVAVYDCA	1818	11	11	79
GSGSKTPRLA	1218	11	12	100
HSGKQCEAL	1400	11	14	86
HSGKQCEVRL	2928	11	11	79
HPIVNSLQGL	2855	11	12	86
HPIVNSLQGL	2250	11	12	86
ITSCSSNSVLA	2816	11	14	100
ITSTVSGFLA	1586	11	11	79
KSTIVPAARLA	1241	11	11	79
LKQKSSQVRL	1243	11	11	79
LKQKSSQVRL	1897	11	11	79
LNSLLTHRLM	2478	11	14	100
LPGQALVGVY	1882	11	11	79
LTCGFQALAGY	126	11	12	86
LTSMLTPSHI	2176	11	13	93
NANVYHQLGV	1418	11	13	93
NINRPPQVNF	14	11	11	79

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IJCV B58 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
PALSPQALIV	1889	11	12	86
PSVAATIGFGA	1261	11	14	100
PTDPRRGRNL	109	11	12	86
PTHYVPSDAM	1936	11	12	86
PTLHGPTLLY	1621	11	11	79
PTPLVRLGV	1628	11	13	93
QMETAGARLV	1320	11	12	86
QAPPSNDQMV	1662	11	12	79
QIVHSLDPF	1462	11	11	86
RCQKHQFHKA	55	11	13	93
RSQLEVTSM	1655	11	11	79
SSASLSAPSL	2208	11	13	83
SSDKCTVTHA	1132	11	12	86
STVWQVQDVA	1653	11	11	86
STVWQVQDVA	2852	11	11	78
TSITGRQKQV	1050	11	12	86
TSIWLWAGVL	1662	11	11	86
TTLPALSTGL	685	11	26	108
VATLIGFGAM	1263	11	11	100
VAGALVAFNM	1864	11	12	86
VAFVWVESDM	974	11	11	79
VAYDAIVCAHA	1420	11	14	100
VAYTRLDQSV	1661	11	12	86
VTSIWLWAGV	76	11	12	86
WVAGVTHWLY	2873	11	11	78
WAKNLRTHF	1248	11	11	78
YAGKTRKQSE	164	11	12	86
YVAGKTRKQSE	1106	11	11	78
YVAGKTRKQSE	288	11	11	78

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Table XIV

NGGVYLL

PCV B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Consistency [%]
KKALVQIV	2825	8	12	86
KPRALIV	2808	8	12	88
KQKAGL	1794	8	12	86
KVPAAVAA	1244	8	11	79
KLENALIV	2235	8	12	86
KIKNGSW	414	8	11	79
KLALISQL	178	8	12	86
KLAPITAY	1030	8	14	100
KLADAVY	729	8	13	93
LYNLGVY	1629	8	13	93
LYNGTV	133	8	11	79
LPALISGL	687	8	14	100
LPQCSRI	168	8	13	93
LPPEPRL	37	8	13	93
LPQCHL	1554	8	13	93
LPEDKML	1720	8	12	86
LPQCKMLV	2761	8	12	86
LVADGVY	1851	8	12	86
LVQKGL	1687	8	12	86
LVQDGLA	1257	8	14	100
LVNPLVY	1884	8	11	79
LVNPLPI	1137	8	12	86
LVYTHQV	1897	8	11	79
LVGVGCA	2773	8	11	79
LVVCESA	2876	8	12	86
MLTDPSSII	2179	8	14	100
MLLSGVNA	1815	8	12	86
NWQDQYL	700	8	12	86
NLVWQED	2239	8	12	86
NPSVANTL	1260	8	14	100
PLGGAAHL	143	8	11	79
PLVNLGA	1628	8	13	93
PPSVNCA	1805	8	12	86
PPSVNCAV	1808	8	11	79
PPVNSQAL	2318	8	11	79
QVNLV	230	8	12	86
QVLENA	336	8	12	86
QVSTQL	2808	8	12	79
QVSTQL	78	8	12	86
RVLGSLVF	2918	8	12	86
RLVHPL	2811	8	11	79
RLILALTA	1029	8	12	88
RLVVLATA	1347	8	12	88
RLVMDVMM	317	8	12	86

HCV B62 Super Motif

[illegible]

IICY B62 Super Mollit

Sequence	Position	No. of Amino Acids	Frequency	Consistency [%]
APPSPWDM	1504	9	12	85
APTLWABM	2859	9	11	79
ACQSYVAL	1251	9	12	86
ANQWNRFL	77	9	100	100
CKSAQLEW	1817	9	14	79
ELCSNHLV	1653	9	11	79
ELVSTW	227	9	12	86
ELMSVRLV	1657	9	11	79
ELVNLPL	1032	9	11	78
ELVNLPL	1883	9	11	78
ELVWESA	2072	9	12	86
ELVYTRHA	1134	9	11	79
EPPLSDGSW	2410	9	12	88
EPPLSRNL	111	9	12	88
EPPLVGAH	1377	9	12	86
EVKSNINW	2245	9	12	86
EVSTINW	1659	9	14	100
EVSTINW	1773	9	12	86
FSHSDIA	177	9	12	86
FLDLDARY	1853	9	13	79
FGVNRQGV	2646	9	11	100
FGVNRQGV	1333	9	14	93
GLPNOCHL	1552	9	13	79
GLRLQYLV	568	9	11	93
GLTHQDAF	1569	9	12	86
GRDEKQW	1812	9	14	100
GPRLVYRL	1853	9	13	93
GVNGSVL	28	9	12	86
GVMAQLVF	1863	9	12	86
GVMAQLVF	1670	9	11	79
GVMAQLVF	161	9	14	100
GVMAVNLG	2619	9	13	93
GVNVCERNA	154	9	12	86
GVNLEDSV	656	9	13	93
HLKNNQDV	1719	9	11	79
HLPIERESK	1769	9	13	93
HMWRFESK	698	9	79	79
HNKQNDQV	1910	9	11	93
HNKQNDQV	1856	9	13	100
ILSRQALV	1891	9	14	100
KKALNRPV	1255	9	14	79
LUSSGSINW	2815	9	11	11
LVFPLQGV	2812	9	8	14
LVFPLQGV	726	9	12	96
LVPLLLADA	1612	9	12	96
LVPLNLSGV	1612	9	12	96

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The concentration of the *Agrobacterium* suspension was 10⁶ cells/ml (a), 10⁷ cells/ml (b), 10⁸ cells/ml (c), and 10⁹ cells/ml (d). The concentration of the *Agrobacterium* suspension was 10⁶ cells/ml (a), 10⁷ cells/ml (b), 10⁸ cells/ml (c), and 10⁹ cells/ml (d).

Sequence

Sequence	Fullion	No. of Amino Acids	Sequence Frequency	Consistency (%)
LPNPGRL	36	9	13	83
LPNPGRL	1888	8	13	93
LPNLSFGA	687	9	12	86
LPNLSFGA	2165	8	12	86
LPNLSFGA	169	9	13	93
LPNLSFGA	1257	9	12	100
LPNLSFGA	1884	9	14	79
LPNLSFGA	1137	9	11	79
LPNLSFGA	1897	9	11	79
LPNLSFGA	1815	9	11	86
LPNLSFGA	1282	9	12	79
LPNLSFGA	700	9	12	86
LPNLSFGA	118	9	12	86
LPNLSFGA	168	9	13	93
LPNLSFGA	1108	9	11	79
LPNLSFGA	143	9	13	93
LPNLSFGA	1628	9	11	79
LPNLSFGA	1605	9	11	79
LPNLSFGA	2317	9	11	79
LPNLSFGA	2807	9	12	86
LPNLSFGA	1534	8	14	100
LPNLSFGA	2857	8	13	93
LPNLSFGA	2310	9	11	79
LPNLSFGA	2808	9	11	86
LPNLSFGA	78	9	12	86
LPNLSFGA	57	9	13	86
LPNLSFGA	1028	9	12	86
LPNLSFGA	2875	9	12	100
LPNLSFGA	2821	8	14	86
LPNLSFGA	2252	9	12	86
LPNLSFGA	156	9	12	100
LPNLSFGA	2178	9	13	93
LPNLSFGA	1893	9	14	79
LPNLSFGA	2831	9	11	79
LPNLSFGA	2849	9	11	79
LPNLSFGA	99	9	11	86
LPNLSFGA	1455	9	12	79
LPNLSFGA	2590	9	11	79
LPNLSFGA	1822	9	11	79
LPNLSFGA	686	9	12	86
LPNLSFGA	125	9	11	79
LPNLSFGA	2871	9	13	93
LPNLSFGA	1827	9	13	93

Sequence	Position		No. of Amino Acids	Sequence Frequency	Consistency (%)
TMDOHETA	1336		9	14	100
WDTITGFE	132		9	12	86
MEDEKANY	157		9	12	86
WUOLQJAY	1852		9	11	79
WVQSGJLA	1666	24.0075	9	12	86
WVWPFSA	1296	22.0272	9	14	100
WOMANIELA	1918		9	14	100
WGVVCKAN	1398		9	11	79
WVTSYMLV	1860	1.0823	9	12	86
WMMRLIFA	1920	24.0073	9	14	100
WMLVQSLA	1665	40.0075	9	12	86
WPLVGAFL	136	1.0817	9	11	79
WLVVQCATV	1590	1.0127	9	12	86
WLVTFHADV	1136	1.0119	9	12	86
WVATVCAHA	1594		9	13	93
WVQKDSV	276	1.0100	9	12	86
WVGGRTFL	637	1.0107	9	13	83
WVSEKMA	1730		9	12	86
WVLSKLVV	1800	24.0101	9	12	86
WLVVGVGCA	1886		10	12	78
WVPPVQKQW	1604	15.0233	10	11	79
WPLVWMLRL	2669	15.0247	10	11	79
WQPGVPMFL	77		10	12	86
WQPGVPMFL	1419	1.0486	10	14	100
WAVVYRIQV	1188		10	11	79
WVCTRGVAA	1917		10	14	100
WVOMANRULA	2941	1.0510	10	12	86
WLPRLGPFLL	1462	1.0487	10	12	86
WVOTIVQFL	1855	1.0495	10	11	79
WLVAVGAGV	1657	1.0490	10	12	86
WLVETVSTV	2617		10	13	93
WLVGVNCEAM	2412	1.0499	10	11	79
WLVDSQSVT	1883	1.0891	10	11	79
WLVMLPALL	1339		10	12	88
WDMETAGHLL	21	1174.01	10	12	88
WDMTHGGGA	2814	1.0506	10	14	100
WLVVQSLA	1666		10	12	86
WLVVWMLRL	1800	1.0481	10	12	86
WVSAEHLV	2921	1.0580	10	12	78
WLVSTVGPFA	1782		10	11	100
WLVTHDAFL	1569	1.0488	10	14	93
WLVGVNCEAM	1912	15.0240	10	12	86
WGVVGVKML	28		10	13	93
WGVVTVYVGA	1081		10	11	79
WVGVCEKML	2819	1.0504	10	14	100

100

1. *1990* *1991* *1992* *1993* *1994* *1995* *1996* *1997* *1998* *1999* *2000* *2001* *2002* *2003* *2004* *2005* *2006* *2007* *2008* *2009* *2010* *2011* *2012* *2013* *2014* *2015* *2016* *2017* *2018* *2019* *2020* *2021* *2022* *2023* *2024* *2025* *2026* *2027* *2028* *2029* *2030* *2031* *2032* *2033* *2034* *2035* *2036* *2037* *2038* *2039* *2040* *2041* *2042* *2043* *2044* *2045* *2046* *2047* *2048* *2049* *2050* *2051* *2052* *2053* *2054* *2055* *2056* *2057* *2058* *2059* *2060* *2061* *2062* *2063* *2064* *2065* *2066* *2067* *2068* *2069* *2070* *2071* *2072* *2073* *2074* *2075* *2076* *2077* *2078* *2079* *2080* *2081* *2082* *2083* *2084* *2085* *2086* *2087* *2088* *2089* *2090* *2091* *2092* *2093* *2094* *2095* *2096* *2097* *2098* *2099* *2100* *2101* *2102* *2103* *2104* *2105* *2106* *2107* *2108* *2109* *2110* *2111* *2112* *2113* *2114* *2115* *2116* *2117* *2118* *2119* *2120* *2121* *2122* *2123* *2124* *2125* *2126* *2127* *2128* *2129* *2130* *2131* *2132* *2133* *2134* *2135* *2136* *2137* *2138* *2139* *2140* *2141* *2142* *2143* *2144* *2145* *2146* *2147* *2148* *2149* *2150* *2151* *2152* *2153* *2154* *2155* *2156* *2157* *2158* *2159* *2160* *2161* *2162* *2163* *2164* *2165* *2166* *2167* *2168* *2169* *2170* *2171* *2172* *2173* *2174* *2175* *2176* *2177* *2178* *2179* *2180* *2181* *2182* *2183* *2184* *2185* *2186* *2187* *2188* *2189* *2190* *2191* *2192* *2193* *2194* *2195* *2196* *2197* *2198* *2199* *2200* *2201* *2202* *2203* *2204* *2205* *2206* *2207* *2208* *2209* *2210* *2211* *2212* *2213* *2214* *2215* *2216* *2217* *2218* *2219* *2220* *2221* *2222* *2223* *2224* *2225* *2226* *2227* *2228* *2229* *2230* *2231* *2232* *2233* *2234* *2235* *2236* *2237* *2238* *2239* *2240* *2241* *2242* *2243* *2244* *2245* *2246* *2247* *2248* *2249* *2250* *2251* *2252* *2253* *2254* *2255* *2256* *2257* *2258* *2259* *2260* *2261* *2262* *2263* *2264* *2265* *2266* *2267* *2268* *2269* *2270* *2271* *2272* *2273* *2274* *2275* *2276* *2277* *2278* *2279* *2280* *2281* *2282* *2283* *2284* *2285* *2286* *2287* *2288* *2289* *2290* *2291* *2292* *2293* *2294* *2295* *2296* *2297* *2298* *2299* *2300* *2301* *2302* *2303* *2304* *2305* *2306* *2307* *2308* *2309* *2310* *2311* *2312* *2313* *2314* *2315* *2316* *2317* *2318* *2319* *2320* *2321* *2322* *2323* *2324* *2325* *2326* *2327* *2328* *2329* *2330* *2331* *2332* *2333* *2334* *2335* *2336* *2337* *2338* *2339* *2340* *2341* *2342* *2343* *2344* *2345* *2346* *2347* *2348* *2349* *2350* *2351* *2352* *2353* *2354* *2355* *2356* *2357* *2358* *2359* *2360* *2361* *2362* *2363* *2364* *2365* *2366* *2367* *2368* *2369* *2370* *2371* *2372* *2373* *2374* *2375* *2376* *2377* *2378* *2379* *2380* *2381* *2382* *2383* *2384* *2385* *2386* *2387* *2388* *2389* *2390* *2391* *2392* *2393* *2394* *2395* *2396* *2397* *2398*

HCY B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
PROTOSSESL	1126	10	11	79
TPLLRLGAV	1627	10	93	86
TPMSWLSIN	2856	10	12	86
TVPSLDLFF	1466	10	12	86
VDITLGGFA	1322	10	12	86
VIALALAYCL	1671	10	12	86
VLDKNETMSA	1337	10	14	100
VLDKNETMSA	1258	10	11	79
VLDKNETMSA	2737	10	12	86
VLDKNETMSA	1666	10	14	100
VLDKNETMSA	1256	10	11	78
VLDKNETMSA	2639	10	12	86
VLDKNETMSA	1940	10	14	100
VLDKNETMSA	1818	10	11	78
VLDKNETMSA	1898	10	12	86
VLDKNETMSA	1665	10	12	86
VLDKNETMSA	1185	10	13	93
VLDKNETMSA	1313	10	11	79
VLDKNETMSA	1136	10	12	86
VLDKNETMSA	276	10	11	79
VLDKNETMSA	1898	10	11	78
VLDKNETMSA	1235	10	13	93
VLDKNETMSA	1602	10	11	78
VLDKNETMSA	2889	10	12	86
VLDKNETMSA	1188	10	11	78
VLDKNETMSA	1917	10	14	100
VLDKNETMSA	1855	10	11	78
VLDKNETMSA	1657	10	12	86
VLDKNETMSA	2617	10	13	93
VLDKNETMSA	132	10	11	78
VLDKNETMSA	1134	10	12	86
VLDKNETMSA	1339	10	11	78
VLDKNETMSA	21	10	12	86
VLDKNETMSA	1721	10	12	86
VLDKNETMSA	1172	10	11	79
VLDKNETMSA	1304	10	14	108
VLDKNETMSA	24	10	11	79
VLDKNETMSA	2646	10	11	79
VLDKNETMSA	1778	10	14	100
VLDKNETMSA	1552	10	12	86
VLDKNETMSA	1782	10	11	79
VLDKNETMSA	1625	10	13	93
VLDKNETMSA	507	10	13	93
VLDKNETMSA	1670	10	12	86
VLDKNETMSA	2619	10	14	100

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HCV B62 Super Motif

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)
SPTHTPESIA	1835	11	12	86
SQLCEPEPV	2153	11	12	86
SVNATIGFGAY	21882	11	14	100
TLGGLYNISKA	13865	11	12	86
TLNLNGGV	1811	11	12	79
TPCIGSSGLY	11725	11	13	83
TPQIPCCDIL	1550	11	12	88
TPNSMLGNI	2856	11	12	86
TLDOENIAGA	1336	11	11	79
VLCEOTINGCA	1521	11	11	79
VLCEOTINGCA	1852	11	12	86
VLDOUATIGA	1565	11	11	79
VLDOUATIGA	2500	11	14	100
VOMNRIIRFA	1918	11	11	78
VWCAILIRIV	1801	11	12	88
WLVGGUALL	1655	11	12	86
YLKSSSGEILL	1185	11	12	86
YLAVNCAITCA	1580	11	12	79
YQNTYCAHICA	1584	11	11	86
YWGGLSSNFI	2716	11	12	86
YVPESDAANY	1593	11	12	86
426				

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ACY A01 Motif with Binding Information

Sequence	Position		No. of Amino Acids	Sequence Frequency	Consistency (%)	A*0.101
ASFCGSPY	166	26.0026	0	20	100	
DNVSLNRY	737	20.0255	10	18	90	0.0001
FAAFPTCCGY	631	20.0254	10	19	95	0.0680
GF-AAFPTCCGY	630		11	19	95	
GRETLEY	140		0	16	85	
GYSLNEMK	579	2.0058	9	17	100	0.1100
HTNWKAGLY	149	1069.04	10	20	95	0.0001
KQAFSTFY	653	20.0256	10	19	85	12.0000
LUDTASALY	30	1069.01	9	17	95	0.0160
LSLDSAAFY	415	1090.07	10	19	95	
LTGHEHLEY	137		11	15	75	
MMWYWGPGFLY	360	1039.01	10	17	95	0.0810
MSITDLEY	103	2.0126	9	15	75	0.6500
NSVLSNRY	738	2.0123	9	18	90	0.0005
PLDKGKPY	124	1147.12	9	20	100	
PLDKGKPY	124	1069.03	10	20	100	0.1700
PTTGITSLY	797	1090.09	9	17	65	0.2100
SASFCGSPY	165		9	20	100	
SLSVSAFY	416	1069.02	9	19	95	5.2000
STTDLEY	104		0	15	75	
TTGITSLEY	798	26.0030	0	17	85	
WLSLDSAAFY	414	26.0551	11	19	95	
WMMWYWGPGS	359	1039.06	11	17	95	0.3200
YPLNAPLY	640	19.0014	8	19	95	
YSLNEMKY	500	26.0032	8	17	85	
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Table XVI

HCY A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A-0301
AAQWNTGER	647	10	12	86	0.0003
AAKALAHGVH	147	10	11	79	
AATLFGA	1264	8	14	100	
AATLFGAY	1264	9	14	100	
AAVCTRGVA	1187	9	11	79	
AAVCTRGVAK	1187	10	11	79	
AAVCTRGVAKA	1187	11	11	79	
AAVCTRGVAKA	1187	11	12	86	
AAVCTRGVAKA	648	9	11	79	
ADGCGSSGAV	1306	10	11	79	
ADVPVRR	1142	8	12	86	0.0260
ADVPVRR	1142	9	11	79	
ADVPVRR	1142	9	11	100	
AFASQNH	1926	8	14	86	
AGALVAK	1865	8	12	86	
AGALVAK	1344	9	12	86	
AGALVLA	1344	11	11	70	
AGALVLA	1781	11	11	100	
AGALVLPNPA	1862	9	12	86	
AGVAGALVA	1862	10	12	86	
AGVAGALVAF	1862	11	12	86	0.0003
AGVAGALVAF	9	9	12	86	
AGWLSRR	94	11	12	86	
AGWLSRR	94	11	12	86	
AGWLSRR	1848	8	12	86	
AGWLSRR	1848	10	12	86	
AGWLSRR	1737	8	12	86	
AGWLSRR	609	8	12	86	
AGWLSRR	609	10	12	86	
AGWLSRR	1806	9	11	78	
ALIVGVCA	1896	10	11	79	0.0003
ALIVGVCA	1793	8	11	79	
ASLMAETL	2208	10	11	79	
ASQLSAPSLK	2208	11	11	79	
ASQLSAPSLK	2208	11	12	86	
ASQLSAPSLK	1928	11	12	86	
ASQLSAPSLK	2204	10	13	100	
ASSASQLSA	165	10	13	93	
ATQNLPCQSF	1265	8	14	100	
ATLFGAY	1265	11	12	86	
ATLFGAYMSK	1265	11	11	79	0.0260
ATRKISR	48	8	11	79	
ATVCAQAQ	1596	9	11	79	
AVCTRGVA	1188	8	11	79	
AVCTRGVAK	1188	9	11	79	
AVCTRGVAKA	1188	10	11	100	
AVQWNRLLA	1917	10	14	100	
AVQWNRLLA	1917	11	14	100	
AVQWNRLLAF	1917	11	14	100	
CAALHSH	1903	8	13	93	

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HCV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A 0.301
CAWVELTPA	1530	9	11	79	
CGFKAMVEY	128	9	13	93	
CGNNTLTCY	2742	8	11	70	
CGSSDLYVTH	1130	11	11	100	
CGPRICRPA	2727	8	11	100	
CGRLGVPLH	2841	11	14	86	
CGSIFLLA	172	9	14	100	
CGSNVSVA	2819	8	14	100	
CGSNVSMH	2819	8	12	86	
CGCSSSLY	1128	9	11	79	0.0001
CTGGVAKA	1190	11	11	79	
CTGGVAKAYDF	1190	11	11	79	
CTMANSTGF	555	11	11	79	0.7600
CTMANSTGFTK	555	11	11	79	0.0008
CAPEKQGH	2599	9	11	79	0.0011
CAPEKQSPK	2599	8	12	86	
CAVTONDF	1462	8	14	100	0.0003
CAHFLSOTK	1574	10	11	79	
DAWLVCESA	2771	11	11	100	
DFSLPFT	1468	8	11	79	
DOGQSSQAV	1400	9	11	79	
DOGQSSQAV	1307	9	11	86	
DOICRST	1316	8	12	86	
DOICRST	1855	11	11	93	0.0003
DLKQVGGVA	1855	11	11	93	
DLKQVNCER	2817	11	13	83	
DLKQVNCERAA	2817	11	11	79	
DLKQVPLVGA	132	6	11	79	
DLVNLPA	1883	8	11	79	
DLVLCESA	2772	8	12	86	0.0003
DLVLTTH	1134	9	12	86	
DLVLTTHA	1134	8	12	86	
DLTLCGFA	124	8	11	79	
DLPVPRH	143	8	14	100	
EAATVYSA	2734	8	14	100	
ECDYKQCAW	1534	10	11	79	
EDVNLPLA	1882	9	11	78	0.0004
EGANMKH	1915	8	14	100	
EPHTGSA	1377	8	13	93	
EQGSSVLA	2245	8	12	86	
ETKQVNLVA	1342	11	12	86	
ETKQVNSVF	1207	9	12	86	
ETKQVNSVF	2596	9	12	86	
FOGPRGKGA	2598	10	11	79	0.0008

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Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

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[illegible]

HCY A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A 0.301
LIECHSK	1398	8	14	100	
LIECHSK	1398	9	14	100	0.5400
LIECHSK	1414	9	11	79	
LIECHSK	1414	10	11	79	0.0003
LIECHSK	2612	10	14	100	
LIECHSK	1030	8	14	100	0.0016
LIECHSK	726	9	14	100	
LIECHSK	726	10	14	100	
LIECHSK	1812	11	12	86	
LIECHSK	1887	10	13	93	0.0003
LIECHSK	1887	8	13	93	
LIECHSK	36	8	12	86	
LIECHSK	97	0	12	86	
LIECHSK	10	10	11	79	0.0002
LIECHSK	2922	9	11	79	
LIECHSK	2211	8	11	79	
LIECHSK	2479	8	12	86	
LIECHSK	2479	9	12	86	0.0003
LIECHSK	690	9	12	86	
LIECHSK	1783	9	14	100	
LIECHSK	126	11	12	86	
LIECHSK	2180	8	14	100	
LIECHSK	1570	8	14	100	
LIECHSK	2176	10	13	93	
LIECHSK	1591	11	12	86	
LIECHSK	1591	11	11	79	
LIECHSK	1591	0	11	79	
LIECHSK	1853	8	11	79	
LIECHSK	1853	10	12	86	
LIECHSK	1667	8	12	86	
LIECHSK	1667	10	12	86	
LIECHSK	1667	11	12	86	
LIECHSK	1257	8	14	100	
LIECHSK	1257	9	14	100	
LIECHSK	1897	8	11	79	
LIECHSK	1897	9	11	79	
LIECHSK	2773	8	11	79	
LIECHSK	2668	11	11	79	
LIECHSK	2668	10	11	79	
LIECHSK	2668	9	11	79	
LIECHSK	134	8	12	86	
LIECHSK	2876	10	14	100	
LIECHSK	2179	9	11	79	
LIECHSK	1	10	11	79	
LIECHSK	1	1	11	79	
LIECHSK	2726	8	11	79	
LIECHSK	2726	9	11	79	
LIECHSK	305	8	11	79	

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ICV A03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
NRSGDY	1772	0	14	100	
NRISQICLA	1772	10	14	100	
NRKQIVY	1080	8	11	79	
NRKQIVYH	1080	9	11	79	
NRKQIVYHGA	1080	11	11	79	
NRIGQWMA	1815	8	12	86	
NRIGQWMA	1815	10	12	86	
NRIRVSEK	2249	9	12	86	0.0010
NRNDQVLY	700	9	12	86	0.0005
NRLPALSRGA	1086	10	13	93	
NLPQSRSEF	1460	10	13	93	
NRCTQITDF	1460	10	12	86	
NRNFRQDCK	14	11	11	79	0.0010
NRNFRQDCK	14	11	11	79	
NRNFRQDCK	1549	11	12	93	
NRNFRQDCK	1089	8	12	86	
PALSTGLH	608	9	12	86	
PALSTGLH	608	11	11	79	
PCSGWMA	1978	10	11	79	
PCGQSSGLY	1127	10	13	93	
PGLGVNCKR	2616	11	11	79	
PGLGVNCKR	1094	8	14	100	
PQCSRSIF	170	11	12	100	
PQCSRSIF	170	11	12	100	
PQCSRSIF	224	0	12	86	
PQCSRSIF	1813	11	13	93	
PQCSRSIF	2932	8	11	79	
PQCSRSIF	1609	11	12	86	
PQCSRSIF	25	9	14	100	
PQCSRSIF	1551	8	13	93	
PQCSRSIF	79	8	14	100	
PQCSRSIF	1295	9	11	79	
PQCSRSIF	1295	10	11	79	
PQCSRSIF	143	8	11	79	
PQCSRSIF	143	10	11	79	
PQCSRSIF	143	11	11	79	
PQCSRSIF	143	11	11	79	
PQCSRSIF	1628	8	13	79	
PQCSRSIF	2667	9	11	79	
PQCSRSIF	2667	11	11	79	
PQCSRSIF	2667	11	13	93	
PQCSRSIF	514	9	14	100	
PQCSRSIF	1261	11	14	100	
PQCSRSIF	1607	8	13	93	
PQCSRSIF	507	9	12	86	
PQCSRSIF	109	9	12	86	0.0008

HCY A03 Motif with Binding Information

[illegible][illegible]

[illegible]

HCV A93 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A10301
TGALGCSF	166	9	13	93	
TGSGKSTK	1237	8	13	93	
TGSGKSTKVPV	1237	11	12	86	
TLMAKNEVF	2590	9	11	79	
TLGFGAMASK	1266	10	12	86	0.0810
TLGFGAMASKA	1266	11	12	86	
TLHGPTLLY	1622	10	11	79	
TLHGPTLLYH	1622	11	11	79	0.0890
TLPALSTGLH	686	11	11	79	
TLVAMMLMTI	2871	11	11	79	
TSCSSNSVAH	2817	10	14	100	
TSCSSNSVAH	2817	11	12	86	
TSEFSGRH	52	8	13	93	
TSEFSGRH	52	10	12	86	0.0003
TSEFSGRH	52	11	12	86	
TSLGDRK	1050	8	12	86	
TSLGDRK	1050	9	13	93	0.0003
TLMAKNEVF	2590	10	11	79	
TLMAKNEVF	2590	11	11	79	
TLMAKNEVF	2590	12	12	86	
TLMAKNEVF	2590	13	11	79	
TLMAKNEVF	2590	14	12	86	
TLMAKNEVF	2590	15	12	86	
TLMAKNEVF	2590	16	12	86	
TLMAKNEVF	2590	17	12	86	
TLMAKNEVF	2590	18	12	86	
TLMAKNEVF	2590	19	12	86	
TLMAKNEVF	2590	20	12	86	
TLMAKNEVF	2590	21	12	86	
TLMAKNEVF	2590	22	12	86	
TLMAKNEVF	2590	23	12	86	
TLMAKNEVF	2590	24	12	86	
TLMAKNEVF	2590	25	12	86	
TLMAKNEVF	2590	26	12	86	
TLMAKNEVF	2590	27	12	86	
TLMAKNEVF	2590	28	12	86	
TLMAKNEVF	2590	29	12	86	
TLMAKNEVF	2590	30	12	86	
TLMAKNEVF	2590	31	12	86	
TLMAKNEVF	2590	32	12	86	
TLMAKNEVF	2590	33	12	86	
TLMAKNEVF	2590	34	12	86	
TLMAKNEVF	2590	35	12	86	
TLMAKNEVF	2590	36	12	86	
TLMAKNEVF	2590	37	12	86	
TLMAKNEVF	2590	38	12	86	
TLMAKNEVF	2590	39	12	86	
TLMAKNEVF	2590	40	12	86	
TLMAKNEVF	2590	41	12	86	
TLMAKNEVF	2590	42	12	86	
TLMAKNEVF	2590	43	12	86	
TLMAKNEVF	2590	44	12	86	
TLMAKNEVF	2590	45	12	86	
TLMAKNEVF	2590	46	12	86	
TLMAKNEVF	2590	47	12	86	
TLMAKNEVF	2590	48	12	86	
TLMAKNEVF	2590	49	12	86	
TLMAKNEVF	2590	50	12	86	
TLMAKNEVF	2590	51	12	86	
TLMAKNEVF	2590	52	12	86	
TLMAKNEVF	2590	53	12	86	
TLMAKNEVF	2590	54	12	86	
TLMAKNEVF	2590	55	12	86	
TLMAKNEVF	2590	56	12	86	
TLMAKNEVF	2590	57	12	86	
TLMAKNEVF	2590	58	12	86	
TLMAKNEVF	2590	59	12	86	
TLMAKNEVF	2590	60	12	86	
TLMAKNEVF	2590	61	12	86	
TLMAKNEVF	2590	62	12	86	
TLMAKNEVF	2590	63	12	86	
TLMAKNEVF	2590	64	12	86	
TLMAKNEVF	2590	65	12	86	
TLMAKNEVF	2590	66	12	86	
TLMAKNEVF	2590	67	12	86	
TLMAKNEVF	2590	68	12	86	
TLMAKNEVF	2590	69	12	86	
TLMAKNEVF	2590	70	12	86	
TLMAKNEVF	2590	71	12	86	
TLMAKNEVF	2590	72	12	86	
TLMAKNEVF	2590	73	12	86	
TLMAKNEVF	2590	74	12	86	
TLMAKNEVF	2590	75	12	86	
TLMAKNEVF	2590	76	12	86	
TLMAKNEVF	2590	77	12	86	
TLMAKNEVF	2590	78	12	86	
TLMAKNEVF	2590	79	12	86	
TLMAKNEVF	2590	80	12	86	
TLMAKNEVF	2590	81	12	86	
TLMAKNEVF	2590	82	12	86	
TLMAKNEVF	2590	83	12	86	
TLMAKNEVF	2590	84	12	86	
TLMAKNEVF	2590	85	12	86	
TLMAKNEVF	2590	86	12	86	
TLMAKNEVF	2590	87	12	86	
TLMAKNEVF	2590	88	12	86	
TLMAKNEVF	2590	89	12	86	
TLMAKNEVF	2590	90	12	86	
TLMAKNEVF	2590	91	12	86	
TLMAKNEVF	2590	92	12	86	
TLMAKNEVF	2590	93	12	86	
TLMAKNEVF	2590	94	12	86	
TLMAKNEVF	2590	95	12	86	
TLMAKNEVF	2590	96	12	86	
TLMAKNEVF	2590	97	12	86	
TLMAKNEVF	2590	98	12	86	
TLMAKNEVF	2590	99	12	86	
TLMAKNEVF	2590	100	12	86	
TLMAKNEVF	2590	101	12	86	
TLMAKNEVF	2590	102	12	86	
TLMAKNEVF	2590	103	12	86	
TLMAKNEVF	2590	104	12	86	
TLMAKNEVF	2590	105	12	86	
TLMAKNEVF	2590	106	12	86	
TLMAKNEVF	2590	107	12	86	
TLMAKNEVF	2590	108	12	86	
TLMAKNEVF	2590	109	12	86	
TLMAKNEVF	2590	110	12	86	
TLMAKNEVF	2590	111	12	86	
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TLMAKNEVF	2590	113	12	86	
TLMAKNEVF	2590	114	12	86	
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TLMAKNEVF	2590	116	12	86	
TLMAKNEVF	2590	117	12	86	
TLMAKNEVF	2590	118	12	86	
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TLMAKNEVF	2590	120	12	86	
TLMAKNEVF	2590	121	12	86	
TLMAKNEVF	2590	122	12	86	
TLMAKNEVF	2590	123	12	86	
TLMAKNEVF	2590	124	12	86	
TLMAKNEVF	2590	125	12	86	
TLMAKNEVF	2590	126	12	86	
TLMAKNEVF	2590	127	12	86	
TLMAKNEVF	2590	128	12	86	
TLMAKNEVF	2590	129	12	86	
TLMAKNEVF	2590	130	12	86	
TLMAKNEVF	2590	131	12	86	
TLMAKNEVF	2590	132	12	86	
TLMAKNEVF	2590	133	12	86	
TLMAKNEVF	2590	134	12	86	
TLMAKNEVF	2590	135	12	86	
TLMAKNEVF	2590	136	12	86	
TLMAKNEVF	2590	137	12	86	
TLMAKNEVF	2590	138	12	86	
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TLMAKNEVF	2590	140	12	86	
TLMAKNEVF	2590	141	12	86	
TLMAKNEVF	2590	142	12	86	
TLMAKNEVF	2590	143	12	86	
TLMAKNEVF	2590	144	12	86	
TLMAKNEVF	2590	145	12	86	
TLMAKNEVF	2590	146	12	86	
TLMAKNEVF	2590	147	12	86	
TLMAKNEVF	2590	148	12	86	
TLMAKNEVF	2590	149	12	86	
TLMAKNEVF	2590	150	12	86	
TLMAKNEVF	2590	151	12	86	
TLMAKNEVF	2590	152	12	86	
TLMAKNEVF	2590	153	12	86	
TLMAKNEVF	2590	154	12	86	
TLMAKNEVF	2590	155	12	86	
TLMAKNEVF	2590	156	12	86	
TLMAKNEVF	2590	157	12	86	
TLMAKNEVF	2590	158	12	86	
TLMAKNEVF	2590	159	12	86	
TLMAKNEVF	2590	160	12	86	
TLMAKNEVF	2590	161	12	86	
TLMAKNEVF	2590	162	12	86	
TLMAKNEVF	2590	163	12	86	
TLMAKNEVF	2590	164	12	86	
TLMAKNEVF	2590	165	12	86	
TLMAKNEVF	2590	166	12	86	
TLMAKNEVF	2590	167	12	86	
TLMAKNEVF	2590	168	12	86	
TLMAKNEVF	2590	169	12	86	
TLMAKNEVF	2590	170	12	86	
TLMAKNEVF	2590	171	12	86	
TLMAKNEVF	2590	172	12	86	
TLMAKNEVF	2590	173	12	86	
TLMAKNEVF	2590	174	12	86	
TLMAKNEVF	2590	175	12	86	
TLMAKNEVF	2590	176	12	86	
TLMAKNEVF	2590	177	12	86	
TLMAKNEVF	2590	178	12	86	
TLMAKNEVF	2590	179	12	86	
TLMAKNEVF	2590	180	12	86	
TLMAKNEVF	2590	181	12	86	
TLMAKNEVF	2590	182	12	86	
TLMAKNEVF	2590	183	12	86	
TLMAKNEVF	2590	184	12	86	
TLMAKNEVF	2590	185	12	86	
TLMAKNEVF	2590	186	12	86	
TLMAKNEVF	2590	187	12	86	
TLMAKNEVF	2590	188	12	86	
TLMAKNEVF	2590	189	12	86	
TLMAKNEVF	2590	190	12	86	
TLMAKNEVF	2590	191	12	86	
TLMAKNEVF	2590	192	12	86	
TLMAKNEVF	2590	193	12	86	
TLMAKNEVF	2590	194	12	86	
TLMAKNEVF	2590	195	12	86	
TLMAKNEVF	2590	196	12	86	
TLMAKNEVF	2590	197	12	86	
TLMAKNEVF	2590	198	12	86	
TLMAKNEVF	2590	199	12	86	
TLMAKNEVF	2590	200	12	86	
TLMAKNEVF	2590	201	12	86	
TLMAKNEVF	2590	202	12	86	
TLMAKNEVF	2590	203	12	86	
TLMAKNEVF	2590	204	12	86	
TLMAKNEVF	2590	205	12	86	
TLMAKNEVF	2590	206	12	86	
TLMAKNEVF	2590	207	12	86	
TLMAKNEVF	2590	208	12	86	
TLMAKNEVF	2590	209	12	86	
TLMAKNEVF	2590	210	12	86	
TLMAKNEVF	2590	211	12	86	
TLMAKNEVF	2590	212	12	86	
TLMAKNEVF	2590	213	12	86	
TLMAKNEVF	2590	214	12	86	
TLMAKNEVF	2590	215	12	86	
TLMAKNEVF	2590	216	12	86	
TLMAKNEVF	2590	217	12	86	
TLMAKNEVF	2590	218	12	86	
TLMAKNEVF	2590	219	12	86	
TLMAKNEVF	2590	220	12	86	
TLMAKNEVF	2590	221	12	86	
TLMAKNEVF	2590	222	12	86	
TLMAKNEVF	2590	223	12	86	
TLMAKNEVF	2590	224	12	86	
TLMAKNEVF	2590	225	12	86	
TLMAKNEVF	2590	226	12	86	
TLMAKNEVF	2590	227	12	86	
TLMAKNEVF	2590	228	12	86	
TLMAKNEVF	2590	229	12	86	
TLMAKNEVF	2590	230	12	86	
TLMAKNEVF	2590	231	12	86	
TLMAKNEVF	2590	232	12	86	
TLMAKNEVF	2590	233	12	86	
TLMAKNEVF	2590	234	12	86	
TLMAKNEVF	2590	235	12	86	
TLMAKNEVF	2590	236	12	86	
TLMAKNEVF	2590	237	12	86	
TLMAKNEVF	2590	238	12	86	
TLMAKNEVF</					

[illegible]

HCY Δ03 Motif with Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*0301
WRTPTRR	107	8	12	85	
WRTPTRR	107	9	12	86	
WRTPTRR	107	11	12	86	
WRTPTRR	107	11	12	86	0.0008
WRTPTRR	96	9	12	86	
WRTPTRR	1920	8	14	100	0.0003
WRTPTRR	1920	9	14	100	
WRTPTRR	1920	11	14	100	0.0530
WRTPTRR	1920	11	14	100	
WRTPTRR	557	9	11	79	
WRTPTRR	557	9	12	86	
WRTPTRR	1665	9	12	86	
WRTPTRR	1665	10	12	86	
WRTPTRR	1665	11	12	86	
WRTPTRR	164	11	11	79	
WRTPTRR	1528	8	11	79	
WRTPTRR	1528	10	12	86	
WRTPTRR	1315	10	12	86	
WRTPTRR	1060	11	12	86	
WRTPTRR	1660	11	12	86	
WRTPTRR	2644	10	11	79	0.0054
WRTPTRR	2644	10	11	79	
WRTPTRR	35	9	13	83	
WRTPTRR	1590	11	12	86	
WRTPTRR	1590	11	11	79	
WRTPTRR	2930	10	11	79	
WRTPTRR	2930	10	11	79	
WRTPTRR	2930	10	11	79	
WRTPTRR	2648	9	12	86	
WRTPTRR	1298	9	12	86	
WRTPTRR	276	10	12	100	
WRTPTRR	637	8	11	100	
WRTPTRR	1939	11	12	86	
WRTPTRR	1939	10	12	86	
WRTPTRR	1939	10	12	86	
WRTPTRR	1939	3	12	86	0.0003
WRTPTRR	567	3			

[illegible]

HCY All Molt With Binding Information

[illegible]

HCY All Mofr With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy [%]	A*1101
FGVAMSKAH	1269	9	12	86	0.0005
FGVGMGDRA	2554	8	12	86	
FILLADAH	728	8	14	100	
FTEAMTRY	2792	8	14	100	
FTGLTHDAA	1567	10	13	93	0.0140
GAAPALAH	146	8	11	79	
GAAPALAHGDR	146	8	11	79	
GAUONNAR	1916	11	11	100	
GAUONNAR	1270	8	14	86	0.0001
GAUAMSKAH	129	8	12	93	
GFADLMGY	129	8	13	86	
GFAMAMSK	1260	8	12	86	
GFAMAMSKAH	2615	10	12	86	0.0010
GFOTYRQON	145	9	11	79	
GGAPALAH	145	9	11	79	
GGCSGQAY	108	8	10	100	
GGCSGQAY	26	8	14	100	0.0001
GGCSGQAY	26	10	14	100	
GGHLEFCH	27	9	14	100	
GGHLEFCH	1392	9	14	100	
GGHLEFCH	1392	11	14	100	0.0010
GGVALLAH	1669	10	12	86	
GGVALLAH	32	8	13	93	
GGVALLAH	32	8	13	93	
GGVALLAH	3027	8	11	79	0.0005
GGVALLAH	1582	8	11	79	
GGVALLAH	1582	8	11	79	
GGVALLAH	1582	8	11	79	
GLAPNAR	2021	8	11	78	0.0005
GLAPNAR	2021	10	11	78	
GLAPNAR	2021	10	11	78	
GLAPNAR	2021	10	11	78	
GLAPNAR	1569	8	12	93	0.0005
GLAPNAR	1569	8	12	93	
GLAPNAR	1569	8	12	93	
GLAPNAR	1569	8	12	93	
GLAPNAR	1931	9	12	86	0.0005
GLAPNAR	1931	9	12	86	
GLAPNAR	1931	9	12	86	
GLAPNAR	1931	9	12	86	
GLAPNAR	2248	11	12	86	0.0005
GLAPNAR	2248	11	12	86	
GLAPNAR	2248	11	12	86	
GLAPNAR	2248	11	12	86	
GLAPNAR	1131	11	12	79	0.0005
GLAPNAR	1131	11	12	79	
GLAPNAR	1131	11	12	79	
GLAPNAR	1131	11	12	79	
GLAPNAR	2641	8	11	79	0.0005
GLAPNAR	2641	8	11	79	
GLAPNAR	2641	8	11	79	
GLAPNAR	2641	8	11	79	
GLAPNAR	2033	10	12	86	0.0005
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0140
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	
GLAPNAR	1083	10	12	86	0.0110
GLAPNAR	1083</				

ICV All Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A-1101
GVVLPRR	33	8	13	93	
GVVLPRRPRR	33	11	13	83	
HADVPRR	1141	8	11	79	
HADVPRR	1141	9	11	79	
HADVPRRPRR	1141	10	11	79	
HARTGSK	1234	8	14	100	
HARTGSKSK	1234	11	13	93	
HELSAFLH	2920	9	11	79	
HOLSAFLSHY	2920	11	11	79	
HPTFLY	1624	9	11	79	
HPTFLYR	1624	11	14	100	
HIDARLSQIK	1572	11	12	100	0.0024
HLIAPGSGK	1232	11	11	79	
HLIKNVDVQY	696	10	14	100	
HLFQSK	1385	8	14	100	
HLFQSK	1385	9	14	100	0.0006
HLFQSKSK	1385	10	13	93	0.0002
HMWMSGQY	1769	10	11	79	
HSTPGCNH	2928	10	11	79	0.0012
HTRPGCNH	222	9	14	100	0.0003
IFASHNH	1925	10	14	100	
IDARLSQIK	1573	8	14	100	
IDARLSQIK	1573	10	14	100	
IFQSKSK	1387	8	12	86	
IFQSKSK	1387	9	11	79	0.0079
IFQSKSK	1415	11	11	79	
IRNSGSH	2250	9	12	86	
IRNSGSH	1286	8	12	86	
IRNDXY	701	0	12	79	0.0044
IRNDXY	701	11	11	79	
IRNDXY	2613	9	13	93	0.0056
IRNDXY	2613	10	13	93	
IRNDXY	30	11	13	86	
IRNDXY	30	11	13	86	
IRNDXY	1404	8	12	86	
KCDLAK	2553	10	12	79	
KFGGADVR	1391	10	11	79	
KGRALFCH	2604	8	12	86	
KGRGPRRA	2604	8	12	86	
KLGAPPLR	25944	8	11	79	
KNGFQCPK	25944	11	12	86	0.0001
KSTKPAAY	1241	8	12	86	
KTKRNTNR	10	8	12	86	
KTKRNTNR	10	9	13	93	0.0100
KTSRQSPR	51	11	12	86	0.0640
KTSRQSPR	51	11	11	79	
KVGGGSGGAY	1305	8	12	86	
LAEDPK	1728	10	12	86	
LDQAEYAGRA	1338	10	12	86	

HCY All Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A*1101
PGGPGDMM	224	8	12	85	
PGGGAGMM	1913	11	13	83	
PGGGAGGV	25	11	11	100	
PGAPGDDH	1951	9	13	93	
PGVPMFLY	79	8	14	100	
PITYSYGK	1295	8	11	79	
PLGGAMALAH	143	11	11	79	
PMGSGYDIH	2687	9	11	79	
PMNTHGTH	1281	8	13	83	
PSPPVAVGTHH	514	11	13	79	
PSMCAAMK	1807	8	11	93	
PTDCHKH	587	8	13	86	0.0005
PTDPRHSH	109	9	12	86	
PTGSGASTK	1236	13	13	93	0.0001
PLTHPPLFLY	1821	11	11	79	
PMVGVTHH	516	9	13	93	0.0005
QAVTGAR	1340	8	12	86	
QMGSVLTHH	29	11	93	93	
QLFTHSH	289	8	12	86	
QLFTSPRH	289	8	11	79	0.0330
QLSAPSLK	2210	8	11	79	
QNDVQY	699	10	11	79	
QNDVQYLY	699	10	11	79	
QNAVCHDPAK	1186	11	11	79	
QNVKSHH	148	8	14	100	
QNVKSHH	447	9	11	79	0.0001
QKNAKSHH	1930	9	12	86	0.0001
QKNAKSHH	1930	10	12	86	
QKNAKSHH	1930	8	13	93	
QKNAKSHH	40-	11	11	79	
QKNAKSHH	40	11	11	83	0.0017
QKNAKSHH	59	9	13	83	
QKNAKSHH	1154	8	12	86	
QKNAKSHH	43	8	11	79	0.0290
QKNAKSHH	43	9	11	79	
QKNAKSHH	2918	11	11	100	
QKNAKSHH	1923	11	14	100	
QKNAKSHH	1923	11	14	79	
QKNAKSHH	2611	11	11	79	0.0270
QKNAKSHH	1029	9	12	86	
QKNAKSHH	635	10	14	100	0.0200
QKNAKSHH	635	10	14	100	
QKNAKSHH	13	11	13	93	
QKNAKSHH	55	8	14	93	
QKNAKSHH	2621	9	12	86	0.5000
QKNAKSHH	156	9	12	86	0.0068

ICV Δ11 Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Conservancy (%)	A:1101
SAEGLISY	2923	8	11	79	
SAEGLISPSLK	2207	11	11	79	
SCSSNSVAH	2818	10	12	86	
SGLVYTR	8	10	12	86	
SGLVYTRI	1133	8	12	86	
SGLVYTRI	1133	9	12	86	
SGKSTVPAAY	1239	11	12	100	
SMALDPHS	2178	0	14	86	
SNSLIPIH	2400	0	12	86	0.0044
SSQLVYTR	1132	9	12	86	
SSQLVYTRH	1132	10	12	86	0.0013
SSNSVAH	2920	0	12	86	
STGLIHL	691	0	12	86	
STKVPAAV	1242	8	12	79	
STVPRCKR	2	8	11	79	
STVPRCKR	2	9	11	79	
STVPRCKR	2	11	11	79	
SVAAALGFQAY	1262	11	14	100	
TCGFQALMGY	127	10	13	93	
TCGFQALMGY	1129	10	11	79	
TCSSSLY	110	8	12	86	
TDPRHGR	1375	9	13	79	0.0001
TGEPHYGK	1588	0	13	93	
TGLTHDAH	1237	0	12	86	0.0610
TGSKGSK	1280	10	11	79	0.0007
TGHPHFLY	1822	11	11	79	
TUHGHTLH	888	11	11	79	
TUPLALGSLH	2071	11	11	79	
TUPLALGSLH	3	8	11	79	
TUPLALGSLH	3	10	11	79	
TUPLALGSLH	3	10	11	79	
TUPLALGSLH	3	11	11	79	
TUPLALGSLH	15	9	11	86	
TUPLALGSLH	2817	11	12	93	
TUPLALGSLH	52	8	13	86	0.0001
TUPLALGSLH	52	10	12	86	
TUPLALGSLH	52	11	12	86	
TUPLALGSLH	1050	0	12	86	
TUPLALGSLH	2177	9	13	93	0.0001
TUPLALGSLH	1263	10	12	100	
TUPLALGSLH	1864	9	11	86	0.0900
TUPLALGSLH	1592	10	12	79	0.0038
TUPLALGSLH	1802	8	11	79	
TUPLALGSLH	1902	9	11	79	
TUPLALGSLH	2622	8	14	100	
TUPLALGSLH	1188	11	11	79	

HCY All Motif With Binding Information

Sequence	Position	No. of Amino Acids	Sequence Frequency	Consensus (%)	A:1101
VDPRTIMH	614	9	13	93	
VDPRTIMV	614	10	13	93	
VDPRTIMY	2597	8	12	86	
VDPKRGCR	2597	11	11	79	
VDPRTIDH	2614	8	11	79	
VDPRTIDH	1566	11	13	93	
VDPRTIDH	1668	11	12	86	
VDPRTALAY	31	9	13	93	0.0019
VDPRTALH	31	10	13	93	
VDPRTALH	31	10	11	79	0.0100
VDPRTALH	3026	9	11	79	
VDPRTALH	1099	10	11	79	
VDPRTALH	1099	11	11	79	
VDPRTALH	1071	8	12	86	
VDPRTALH	1327	11	12	86	
VDPRTALH	157	8	12	86	
VDPRTALH	2175	11	13	93	
VDPRTALH	1652	9	11	79	
VDPRTALH	2639	10	11	79	
VDPRTALH	1138	11	11	79	0.0005
VDPRTALH	1901	8	11	78	
VDPRTALH	1901	9	11	78	
VDPRTALH	1901	10	11	78	
VDPRTALH	1090	11	11	83	
VDPRTALH	517	8	11	83	
VDPRTALH	93	11	12	86	
VDPRTALH	76	11	12	86	
VDPRTALH	2073	8	12	86	
VDPRTALH	107	8	12	86	
VDPRTALH	107	11	12	86	
VDPRTALH	107	11	12	86	0.0005
VDPRTALH	96	14	14	100	
VDPRTALH	1920	9	11	79	0.0610
VDPRTALH	557	8	14	100	
VDPRTALH	1771	8	11	79	
VDPRTALH	1526	10	12	86	
VDPRTALH	1315	10	11	79	
VDPRTALH	2644	9	13	93	0.0005
VDPRTALH	35	8	11	79	
VDPRTALH	2930	8	14	100	
VDPRTALH	637	10	12	86	0.0001
VDPRTALH	1938	3	12	86	

[illegible]

ICV A24 Motif With Binding Information

[illegible]

ICV A24 Motif With Binding Information

Sequence	Position	No of Amino Acids	Sequence Frequency	Conservancy [%]	A-24.01
TMALQAGL	1664	9	12	0.6	
TSYDQK	1287	8	13	0.3	
TSYDQK	1287	9	12	0.6	0.0230
TSYDQK	1287	8	13	0.3	
WLGQTH	1566	1287	11	7.9	
WKSSTG	2639	6	13	9.3	0.0016
WLLPRTPL	34	11	13	9.3	
WMLNLLAF	1820	8	14	100	
WYGLDLSVI	10	10	14	100	
53	1422	2			

[illegible]

Table XIXb.

HCY DR Super Motif With Binding Data

[illegible]

ICY DR Super-Motif With Binding Data

[illegible]

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[illegible]

11CY DR 3A Motif Binding Data Not Included

Cue Sequence	Cue First	Cue Conserving (%)	Emptying Sequence	Emptying In Sequence	Emptying In Polyprotein	Emptying Sequence Frequency	Emptying Sequence Conserving (%)
11ACGCGSA	11	79	YQAT1ACGCGSAQV		1501	10	71
19ADPHTI	14	100	YAPSDPHTIETI		1180	11	79
1LEGEGKD	12	100	YQATLEGEGKDT		2401	11	79
1PERFERV	14	86	QKQATPERFERVAL		2182	9	79
MANQWAKMNV	12	86	GIHMANQWAKMNGRT		315	12	57
MLDPSRT	12	100	LTSLMLDPSRTIET		2176	6	43
YADDAKNTG	11	79	MACSKNDLWVSTW		1651	6	43
WCCDLEEV	12	86	VVVVADLALFQYTG		1427	8	43
WCCDLEEV	12	86	GLPQCDLLEWSEV		1352	10	71
VFTDQISPP	11	79	HEPHTDQISPPV		2811	11	79
VFTDQISPP	11	79	YQATVFTDQISPPV		1211	10	71
VCECTQVA	12	93	YQATVCECTQVAG		1510	12	88
VCECTQVA	12	93	GVNVLCECTQVAG		154	10	71
VLDVLAAY	11	79	IGNVLVLAAYQVG		1849	11	79
KQDQKQKQ	11	83	VQDQKQKQNTV		2597	11	79
YQRTKQKQ	11	83	QYQRTKQKQNTV		2808	11	79
YQRTKQKQ	11	79	QYQRTKQKQNTV		2802	8	57
YQRTKQKQ	12	80	GNVYQRTKQKQNTV		1938	12	88
YVPESDMA	12	80	PLHNVYPESDMAV				

Table XXC IICV JB Moll

Core Sequence	Core Freq	Core Consensus (%)	Example Sequence	Position in IICV Protein	Example Sequence Frequency	Example Sequence Consensus (%)
ITD SGKCD	14	100	ITD SGKCD	1395	14	100
PSGKPCD	11	79	PSGKPCD	2667	11	79
LEGTGKA	12	78	LEGTGKA	1726	6	71
LEPTLIRT	11	78	LEPTLIRT	1816	10	71
WQVWVSS	11	78	WQVWVSS	43	10	71
WQVWVSS	12	86	WQVWVSS	1123	11	79
LETRVGR	11	79	LETRVGR	1		

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Table XXd HCV 3B Motif Binding Data

Core Sequence	Exemplary Sequence	DR1	DR2-DR1	DR2-DR2	DR3	DR4-4	DR5-5	DR5-11	DR6-12	DR6-19	DR7-2	DR7	DR8	DR4-3
RD RANCA RD RANCA LAERPNKA LPPNKEPT VLAIRKISE VLIVRIADK KSTIVPCKI ?	HAKSHVPCSEA PAQESGPRGPGIV QMQLAEQNOVAL LRLVPLIDPTTL ILGVANIKESGQ SCLTVNIDNDIPI ?				0.0190 0.0022									

09257727-026000

TABLE XXI. Population coverage with combined HLA Supertypes

HLA-SUPERTYPES	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
a. Individual Supertypes						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	38.6	52.7	48.8	35.5	47.1	44.7
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
b. Combined Supertypes						
A2, A3, B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

HIC ANALOGS

AA	Sequence	Fixed	A1	A2	A3	A24	B7	1*
		Numen.	Motif	Super Motif	Super Motif	Motif	Super Motif	Anchor Fixer
9	CNKCKCNIV		N	Y	N	N	N	
	40							

00357737-072000

Table XXIII. Immunogenicity of identified supermotif-bearing peptides

Supermotif	Peptide	Sequence	Protein	Position	Human ^a			Immunogenicity		Transgenic mice ^b	
					Barnabai patients	Barnabai contacts	Chisari	Pape	overall	Frequency	Response
A2	1073.05	LLFNLGGWV	NS4	1812	1/6	7/17	2/21	0/6	10/50	6/6	6.4 (1.7)
	1090.18	FILLADARV	NS1/E2	728	2/6	7/17	1/21	0/6	10/50	5/6	9.5 (3.0)
	1013.02	YLVAAYQATV	NS4	1590	1/6	4/17	1/21	0/6	6/50	5/6	8.5 (3.7)
	1090.22	RLVFPDLGV	NS5	2578	2/6	5/17	0/21	0/6	7/50	0/6	-
	1013.1002	DLMGVPLV	Core	132	2/6	7/17	1/21	1/6	11/50	5/6	8.8 (2.6)
	24.0073	WMANRLAFA	NS4	1920	1/6	3/17	2/21	1/6	7/50	0/6	-
	24.0075	VLVGVLAA	NS4	1666	1/6	6/17	3/21	1/6	11/50	0/6	-
	1174.08	HMMNFSGI	NS4	1769	3/6	3/17	2/21	0/6	8/50	6/6	6.4 (1.7)
	1073.06	LLAGYGACV	NS4	1851	2/6	3/17	0/21	0/6	5/50	3/6	54.7 (3.3)
	1073.07	YLLRRRQPL	Core	35	2/6	5/17	7/21	1/6	17/50	4/6	59.1 (7.2)
A3	24.0071	LLFLLADA	NS1/E2	726	2/6	9/17	0/21	0/6	17/50	0/6	-
	1.0119	YLVTBHADV	NS3	1131	6/6	10/17	0/21	1/6	17/50	0/6	-
	1.0952	KISESQPR	Core	51	2/16	1/4	3/12	0/6	6/38	3/6	23.4 (1.3)
	1073.11	RLGVRATRK	Core	43	4/16	1/4	7/12	1/6	13/38	3/6	42.2 (1.2)
	1.0955	QLFTSPRR	ENV	290	1/16	0/4	6/12	1/6	8/38	2/6	2.8 (1.1)
	1073.13	RMVYGVEHR	NS1/E2	632	5/16	1/4	4/12	1/6	11/38	3/6	4.4 (1.1)
	1.0123	LPCHSKK	NS3	1396	6/16	1/4	4/12	2/6	13/38	3/6	56.5 (1.7)
	1073.10	GVAGALVAFK	NS4	1863	3/16	0/4	6/12	0/4	11/38	6/6	7.1
	24.0090	VAGALVAFK	NS4	1864	4/16	1/4	6/12	0/4	11/38	1/6	-
	24.0086	TLLEFGAYMSK	NS3	1262	6/16	2/12	2/5	2/5	10/33	1/6	-
B7	1145.12	LPQCSFSF	Core	169			2	3/10	5		

Table XXIV. Human and murine MHC-peptide binding assays established using purified MHC molecules and gel filtration chromatography

A Class I binding assays					
Species	Antigen	Allele	Cell line	Source	Radio-labeled peptide
Human					
A1	A*0101	Steinlin	Hu J chain 10>110	YTAAPVPLVY	no VEN in P1 cocktail
A2	A*0201	IF	HBVc 18-27 R6->Y	FLPSDYPRSV	"
A2	A*0202	P815 (transfected)	HBVc 18-27 R6->Y	FLPSDYPRSV	"
A2	A*0203	FDN	HBVc 18-27 R6->Y	FLPSDYPRSV	"
A2	A*0206	CLA	HBVc 18-27 R6->Y	FLPSDYPRSV	"
A2	A*0207	721 221 (transfected)	HBVc 18-27 R6->Y	FLPSDYPRSV	"
A3		GM3107	non-natural (A3CON1)	KVPPYALINK	"
A11	A*2402	BVR	non-natural (A3CON1)	KVPPYALINK	"
A24	A*3101	KAS116	non-natural (A24CON1)	AYIDYVYKRF	"
A31	A*3101	SPACH	non-natural (A3CON1)	KVPPYALINK	"
A33	A*3301	LWAGS	non-natural (A3CON1)	KVPPYALINK	"
A28/68	A*6801	CTR	HBVc 141-151 T7->Y	STLPETVYRR	"
A28/68	A*6802	AMAI	HBVc pol 646-654 (C4->A)	FTQACVYAL	"
B7	B*0702	GM3107	A2 signal seq. 5-13 (L7->Y)	APRLTVYLL	"
B8	B*0801	Steinlin	ILV gp 586-593 Y1->F, Q5->R 608	FRYNGLIHR	"
B27	B*2705	LC2		FRYNGLIHR	"
B35	B*3501	CTR, BVR	non-natural (B35CON2)	FPFKYAAAF	"
B35	B*3502	TS1	non-natural (B35CON2)	FPFKYAAAF	"
B35	B*3503	EHM	non-natural (B35CON2)	FPFKYAAAF	"
B44	B*4403	PITOUT	EF-1 G6->Y	AEKGGYRY	"
B51		KAS116	non-natural (B35CON2)	FPFKYAAAF	"
B53	B*5301	AMAI	non-natural (B35CON2)	FPFKYAAAF	"
B54	B*5401	K73	non-natural (B35CON2)	FPFKYAAAF	"
Cw4	Cw*0401	CTR	non-natural (C4CON1)	QYDDAAYVKL	"
Cw6	Cw*0602	721 221 transfected	non-natural (C6CON1)	YRHDEGNVL	"
Cw7	Cw*0702	721 221 transfected	non-natural (C6CON1)	YRHDEGNVL	"
Mouse					
D ^b		EL4	Adenovirus E1A P7->Y	SGRNVYPEI	"
K ^b		EL4	VSV NP 52-59	RGVYVQGL	"
D ^d		P815	HIV-IIIB ENV G4->Y	RGPVRAVFI	"
K ^d		P815	non-natural (KdCON1)	KENPAKTYI	"
L ^d		P815	HBVc 28-39	IPQSLDSYWTSL	"

Table XXIV. Human and murine MHC-peptide binding assays established using purified MHC molecules and gel filtration chromatography

B. Class II binding assays						
Species	Antigen	Allele	Cell line	Radioabeled peptide		Notes
				Source	Sequence	
Human	DR1	DRB1*0101	LQ2	HA Y307-319	YKPYKONITLKAT	
	DR2	DRB1*1501	L466.1	MBP 88-102Y	VVHFKNVITRPPEY	
	DR2	DRB1*1601	L242.5	non-natural (760.16)	VAIFAAKTAATAFA	
	DR3	DRB1*0301	MAT	MT 65BD Y3-13	VKTAIDPEEAR	
	DR4*04	DRB1*0401	Preiss	non-natural (717.01)	YARFOSQTTTKOKT	optimal assay pH is 4.5
	DR4*10	DRB1*0402	YAR	non-natural (717.10)	YARFOSQTTLKAAA	
	DR4*14	DRB1*0404	BIN 40	non-natural (717.01)	YARFOSQTTTKOKT	
	DR4*15	DRB1*0405	K13	non-natural (717.01)	YARFOSQTTTKOKT	
	DR7	DRB1*0701	Pilot	Tet. tox. 830-843	QYKANSKFTGTE	
	DR8	DRB1*0802	OLL	Tet. tox. 830-843	QYKANSKFTGTE	
	DR8	DRB1*0803	LUTY	Tet. tox. 830-843	QYKANSKFTGTE	
	DR9	DRB1*0901	HID	Tet. tox. 830-843	QYKANSKFTGTE	
	DR11	DRB1*1101	Swieg	Tet. tox. 830-843	QYKANSKFTGTE	
	DR12	DRB1*1201	Herfur	unknown eluted peptide	EALHOLKINPVYS	
	DR13	DRB1*1302	H0301	Tet. tox. 830-843 S>-A	QYKANSKFTGTE	
Mouse	DR51	DRB5*0101	GMD107 or L416.3	Tet. tox. 830-843	QYKANSKFTGTE	
	DR51	DRB5*0201	L255.1	HA 307-319	PKYVAKONTLKAT	
	DR52	DRB3*0101	MAT	Tet. tox. 1272-1284	NOQIGNDPNDRL	
	DR53	DRB4*0101	L257.6	non-natural (717.01)	YARFOSQTTTKOKT	no NEM in Pl mix
	DO3.1	DOA1*0301/DOB1*0301	PF	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	
	IA ^b		DR27.4	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	optimal assay pH is 5.5
	IA ^b		A20	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	
	IA ^b		CH-12	HEL 46-61	YINDGSTVDYGLONSR	optimal assay pH is 5.0
	IA ^b		LS102.9	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	
	IA ^b		91.7	non-natural (ROIV)	YAHAAHAAHAAHAAHAA	
IE ^a		A20	Lambda repressor 12-26	YLEDARRKKAYIEKKK	optimal assay pH is 5.0	
		CH-12	Lambda repressor 12-26	YLEDARRKKAYIEKKK	optimal assay pH is 5.0	

Table XXV. Monoclonal antibodies used in MHC purification.

Monoclonal antibody	Specificity
W6/32	HLA-class I
B123.2	HLA-B and C
IVD12	HLA-DQ
LB3.1	HLA-DR
M1/42	H-2 class I
28-14-8S	H-2 D ^b and L ^d
34-5-8S	H-2 D ^d
B8-24-3	H-2 K ^b
SF1-1.1.1	H-2 K ^d
Y-3	H-2 K ^b
10.3.6	H-2 I A ^k
14.4.4	H-2 IE ^d , IE ^k
MKD6	H-2 I A ^d
Y3P	H-2 I A ^b , I A ^s , I A ^u

Table XXVI: HCV-derived conserved high algorithm A*0201-binding peptides

Peptide	Molecule	1st Position	Sequence	Consv.	A2-super-type binding capacity (IC50 nM)						
					A*0201	A*0202	A*0203	A*0206	A*6802	A2 XRN	
1073.05	NS4	1812	LLFNILGGWV	85	4.2	113	3.2	19	33	5	
1090.18	NS1/E2	728	FLLADARV	92	18	90	149	247	111	5	
1013.02	NS4	1590	VLVAVYQATV	85	20	39	16	82	33	5	
1090.22	NS5	2611	RLVIPPDLGV	79	56	391	10	370	8000	4	
1013.1002	CORE	132	DLMGVRLV	79	80	4778	204	481	12	4	
24.0073	NS4	1920	WMNRLAFA	100	122	130	3.3	1609	400	4	
24.0075	NS4	1666	VLVGGVLA	85	185	331	32	308	3077	4	
1174.08	NS4	1769	HMWNEISG	92	15	10750	77	132	7547	3	
1073.06	NS4	1851	ILAGYGAGV	79	116	143	5.0	755	889	3	
1073.07	CORE	35	YLLPRKGPRL	92	125	6143	455	416	10256	3	
24.0071	NS1/E2	726	LLFILLADA	100	217	287	455	3364	3077	3	
1.0119	LORF	1131	YLVTRHADV	85	455	2048	3.6	71	3077	3	
24.0065	NS4	1891	ILSPGALVV	92	238	10750	27	1028	3077	2	
1013.12	NS1/E2	686	ALSTGLIHL	85	313	7167	45	18300	10256	2	
993.14	NS1/E2	696	HLHONIVDV	85	500	3071	19	1370	10811	2	
1090.21	NS5	2918	RLHGILSARSL	79	179	782	625	18300	12500	1	

Table XXVII: HCV-derived conserved high algorithm A*03 and/or A*11 binding peptides

Peptide	Molecule	1st Position	Sequence	Consv.	A3-superlyse binding capacity (IC50 nM)						
					A*03	A*11	A*3101	A*3301	A*6801	A3 XRN	
1 0952	CORE	51	KTSERSQPR	92	69	94	67	1813	145	4	
1073.11	CORE	43	RLGVKATRK	79	12	207	429	-	-	3	
1.0955	ENV1	290	QLFTTSPRR	79	15	182	621	3766	3	3	
1073.13	NS1/E2	632	RMVYGVGEHR	100	15	300	95	9667	1778	3	
1.0123	NS3	1396	LIRCHSKKK	100	20	32	2355	24167	333	3	
1073.10	NS4	1863	GVAGALVAFK	85	28	4	3273	26364	118	3	
24.0090	NS4	1864	VAGALVAFK	85	46	7	3750	11600	258	3	
24.0086	NS3	1262	LGRGAYMSK	85	136	21	2950	22308	222	3	
1174.16	NS1/E2	557	WNINSTGFTK	79	208	74	12857	690	1429	2	
1073.14	NS3	1261	TLGFGA YM SK	85	136	98	-	22308	8889	2	
1090.23	LORF	1183	AVCTRGVAK	79	423	240	16364	-	-	2	
1090.24	NS5	2596	EYFCVQPEK	85	13750	222	-	-	18	2	
24.0103	NS1/E2	647	AACNWTGIER	85	36667	429	400	5273	4444	2	
1073.16	NS3	1232	HLHAPTGSGK	85	19	2500	-	-	2857	1	
1073.12	NS3	1395	HLIRCHSKKK	100	423	-	20000	-	-	1	
1090.26	NS3	1395	HLIRCHSKKK	100	440	10000	-	-	8000	1	

* A dash indicates IC50nM >30,000

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Table XXVIII: HCV derived conserved B*0702 binding peptides

A. High conservancy 9- and 10-mer peptides.

Peptide	Molecule	1st Position	Sequence	Consv.	B7-supertype binding capacity (IC50 nM)					B7 XRN
					B*0702	B*3501	B*51	B*301	B*3401	
1145.12	Core	169	LPQGSSTF	92	28	90	100	114	6667	4
15.0048	E2	681	LPALSTGIL	85	157	-	2.8	1500	20000	2
15.0234	NS3	1620	KPTLHGPTL	79	3.9	-	27500	-	-	1
15.0247	NS5	2835	APTLWARMIL	79	6.3	-	5500	-	-	1
15.0042	CORE	99	SPRGSRPSW	79	14	-	11000	-	-	1
15.0039	Core	57	QPRGRROPI	92	24	-	-	-	-	1
15.0218	Core	37	LPRRGPRLG	92	29	-	-	-	4000	1
15.0060	NS5	2615	SPGQRVEEL	79	46	-	27500	-	-	1
15.0043	Core	111	DPGRSRNL	85	324	-	-	-	-	1
15.0063	NS5	2835	APTLWARM	79	344	-	4583	-	-	1
1292.17	NS5	2317	PPVHGCP	79	393	-	-	-	-	1
15.0239	NS4	1893	SPGALVGVV	79	423	-	3438	-	-	1
15.0235	NS3	1621	TPILYRLGAV	92	458	-	6875	-	909	1

Table XXVIII: HCV derived conserved B*0702 binding peptides

B. Additional HCV derived B7 supermotif peptides:

Peptide	Molecule	1st Position	Sequence	Conv.	B7-superpeptide binding capacity (IC50 nM)						B7 XRN
					B*0702	B*3501	B*51	B*5301	B*5401	B7 XRN	
29.0035	NS3	1378	IPFYGRAL	92	458	-	46	-	50	3	
29.0040	Core	37	LPRRGRRL	92	0.85	-	306	-	5000	2	
29.0036	Core	137	IPLVGAPL	79	13	2250	79	-	2857	2	
16.0187	NS1/E2	680	LPDSFTTLPA	64	423	24000	9167	-	15	2	
29.0039	Core	169	LPGCSFSI	92	500	200	932	620	6250	2	
15.0219	Core	142	APLGGARAL	71	9.5	-	-	-	12500	1	
29.0031	NS5	2869	APTLWARM	79	13	-	4583	-	4348	1	
15.0231	NS3	1512	RPSGNFDSV	71	153	-	-	-	-	1	
29.0085	NS5	2474	LPINALNSL	57	220	18000	1170	-	11111	1	
29.0037	NS5	2608	KPARLVF	85	367	-	3235	-	16667	1	
15.0237	NS4	1789	NPALASLMAP	71	393	9000	5000	-	-	1	
29.0118	NS5	2869	APTLWARMILM	79	423	-	-	-	3030	1	
29.0042	NS4	1720	LPYTEQGM	85	423	-	1375	-	7692	1	

C. Engineered analogs of B7 supermotif peptides.

Peptide	Molecule	1st Position	Sequence	Conv.	B7-superpeptide binding capacity (IC50 nM)						B7 XRN
					B*0702	B*3501	B*51	B*5301	B*5401	B7 XRN	
1145.12	Core	169	LPGCSHSF	92	28	90	100	114	6667	4	
1292.24	Core	169	LPGCSHSI		37	4364	5.3	262	1056	3	
1145.13	Core	169	FPGCSHSF		19	1.6	132	3.2	6.7	5	

* A dash indicates IC50 nM >30,000.

A. A1-motif peptides

A dash indicates IC50 nM >25000

Peptide	Molecule	Position	Sequence	HLA-A*2402	
				Conserv.	binding (IC50 nM)
24.0092	NS4	1765	FWAKHMWNF	85	1.7
13.0075	NS4	1778	QYLAGLSTL	100	250
1073.18	NS1/E2	636	MYVGGVEHRL	92	444
13.0074	NS3	1297	TYSTYGKFL	85	522
13.0134	NS5	2647	QYSPGQRFVEF	79	667
24.0091	NS4	1772	NFISGIQVL	100	706
13.0131	Core	135	GYIPLVGAPL	79	2105
24.0108	Core	173	SFSIFLLALL	100	2927
13.0132	NS3	1248	AYAAQGYKVL	79	13333
13.0133	NS4	1859	GYGAGVAGAL	85	-
1174.08	NS4	1769	HMWNFISGI	93	
	E1	317	RMAWDDMMMNW	85	
	NS1/E2	635	RMVVGVEHRL	93	
	NS3	1422	YYRGLDVSVI	100	
	NS3	1468	DFSLDPTFTI	100	
	NS3	1608	SWDQMWKCL	79	
	NS3	1664	TWVVLGGVL	85	
	NS4	1732	QFKQKALGL	85	
	NS4	1732	QFKQKALGLL	85	
	NS4	1765	FWAKHMWNFI	85	
	NS4	1919	QWMNRLIAF	100	
	NS5	2241	LWRQEMGGNI	85	
	NS5	2669	GFSYDTRCF	79	
	NS5	2875	RLMLMTHFF	85	

A dash indicates IC50 nM >25000

Table XXX: Immunogenicity of A2-super-type cross-reactive binders

Peptide	Sequence	Protein	Position	Barnaba: Barnaba:				Immunogenicity		
				patients		contacts		Human ^a	Transgenic mice ^b	
				Chisari	Pape	overall	Frequency			Response
1070.05	LLFLINLGGWV	NS4	1812	1/6	7/17	2/21	0/6	10/50	6/6	6.4 (1.7)
1090.18	VILLADARV	NS1/E2	728	2/6	7/17	1/21	0/6	10/50	5/6	5.5 (3.0)
1013.02	YLVAAYQATV	NS4	1590	1/6	4/17	1/21	0/6	6/50	5/6	8.5 (3.7)
1090.22	RLVMEPIV	NS5	2578	2/6	5/17	0/21	0/6	7/50	0/6	-
1013.1002	LLMEYPIV	Core	132	2/6	7/17	1/21	1/6	11/50	5/6	8.8 (2.6)
24.0073	WMNRLIAFA	NS4	1920	1/6	3/17	2/21	1/6	7/50	0/6	-
24.0075	VLVGGVLAIA	NS4	1666	1/6	6/17	3/21	1/6	11/50	0/6	-
1174.08	HMWNISGI	NS4	1769	3/6	3/17	2/21	0/6	8/50	6/6	6.4 (1.7)
1073.06	ILAGYQAGV	NS4	1851	2/6	3/17	0/21	0/6	5/50	3/6	5.4 (7.3)
1073.07	YLLPRGRL	CORE	35	2/6	5/17	7/21	1/6	17/50	4/6	59.1 (7.2)
24.0071	LLFLILLADA	NS1/E2	726	2/6	9/17	0/21	0/6	11/50	0/6	-
1101.19	YLVTIRHADV	NS3	1131	6/6	10/17	0/21	1/6	17/50	0/6	-

a. Data shown represents the number of positive responses over the total number of patients or contacts examined.

b. Frequency represents the number of positive responses over the total number of mice examined. Response indicates the average magnitude (standard deviation) of the response in positive animals, measured in lytic units.

Table XXXI: Immunogenicity of A3-supertype cross-reactive binders

Peptide	Sequence	Protein	Position	Immunogenicity						
				Human ^a				Transgenic mice ^b		
				Barnaba patients	Barnaba contacts	Chiari	Pape	overall	Frequency	Response
1.0952	KTSERSQPR	CORE	51	2/6	1/4	3/12	0/6	6/38	3/6	23.4 (1.3)
1073.11	RLGVRAIRK	CORE	43	4/16	1/4	7/12	1/6	13/38	3/6	42.2 (1.2)
1.0955	QLFTTSPRR	ENV	290	1/16	0/4	6/12	1/6	8/38		
1073.13	RMVVGQVEHR	NS1/E2	632	5/16	1/4	4/12	1/6	11/38	2/6	2.8 (1.1)
1.0123	LIFCHSKKK	NS3	1396	6/16	1/4	4/12	2/6	13/38	3/6	4.4 (1.1)
1073.10	GVAGALVAFK	NS4	1863	3/16	0/4	6/12	2/6	11/38	6/6	56.5 (1.7)
24.0090	VAGALVAFK	NS4	1864	4/16	1/4	6/12	0/4	11/38		
24.0086	TIIGFGAVMSK	NS3	1262	6/16		2/12	2/5	10/33	1/6	7.1

a. Data shown represents the number of positive responses over the total number of patients or contacts examined.

b. Frequency represents the number of positive responses over the total number of mice examined. Response indicates the average magnitude (standard deviation) of the response in positive animals, measured in lytic units.

Table XXXII. Candidate HCV-derived HTL epitopes

Selection criteria	Peptide	Sequence	Source	Conservancy	
				Total	Core
A DR-supermotif conserved 15mers	1283.01	GQIVGGVYLLPRRGPR	HCV Core 28	93	93
	1283.02	VYLLPRRGPRLGVR	HCV Core 34	93	93
	1283.03	GWLLSPRGSRPSWGPT	HCV Core 95	79	79
	1283.04	LKGVIDLTGCGFADL	HCV Core 119	79	86
	1283.05	IDLTTCGFAIDMGVI	HCV Core 123	86	86
	1283.06	ADLMGYIPLVGAPLG	HCV Core 131	79	79
	1283.07	GVRVLEDGVNYATGN	HCV Core 154	86	86
	1283.08	GVNYATGNLPGCSFS	HCV Core 161	79	86
	1283.09	GCSFSIFLLALLSCL	HCV Core 171	86	100
	1283.10	GHRMAWDMMMNWSPT	HCV E1 315	86	86
	1283.11	CGPVYCFPSPPVVG	HCV NS1/E2 506	93	93
	1283.12	VYCFPSPPVVGITD	HCV NS1/E2 509	93	93
	1283.13	GNWFGCTWMNSTGFT	HCV NS1/E2 550	79	86
	1283.14	FTTLPALSTGLIHLH	HCV NS1/E2 684	79	86
	1283.17	DLVLTVRHADVIPVR	HCV NS3 1134	79	79
	1283.18	RAAVCTRGVAKAVDF	HCV NS3 1186	79	79
	1283.20	AQGYKVLVLNPSVAA	HCV NS3 1251	79	100
	1283.21	GYKVLVLNPSVAATL	HCV NS3 1253	100	100
	1283.22	VLVLNPSVAATLGFG	HCV NS3 1256	100	100
	1283.23	GTVLDAQETAGARLV	HCV NS3 1335	86	86
	1283.24	GARLVLATATPPGS	HCV NS3 1345	79	86
	1283.25	GRHLIFCHSKKKCDE	HCV NS3 1393	100	100
	1283.27	DSVIDCNTCVQTQVD	HCV NS3 1454	86	86
	1283.28	TVDFSLDPFTIETT	HCV NS3 1466	79	100
	1283.30	FTGLTHIDAHFLSQT	HCV NS3 1567	93	93
	1283.31	YLVAYQATVCARAQA	HCV NS3 1591	79	93
	1283.32	KPTLHGPTPLLYRLG	HCV NS4 1620	79	79
	1283.33	LEVVTSTWLVGGVL	HCV NS4 1658	86	86
	1283.34	TWLVGGVLAALAAY	HCV NS4 1664	86	86
	1283.35	AEQFKQKALGLLQTA	HCV NS4 1730	86	86
	1283.40	PAILSPGALVGVVCA	HCV NS4 1889	79	93
	1283.41	GAILVGVVCAAILRR	HCV NS4 1895	79	79
	1283.42	CAAILRRHVGFGEQA	HCV NS4 1903	79	79
	1283.43	AVQWMNRLIAFASRG	HCV NS4 1917	100	100
	1283.44	MNRLIAFASRGNHVS	HCV NS4 1921	86	100
	1283.48	ANLLWRQEMGGNITR	HCV NS5 2238	86	86
	1283.49	ROEMGGNITRVESEN	HCV NS5 2243	86	86
	1283.52	ARLIVFPDLGVRVCE	HCV NS5 2610	79	79
	1283.53	FPDLGVRVCEKMAVY	HCV NS5 2615	79	100
	1283.54	GVRVCEKMAVYDVVS	HCV NS5 2619	79	100
	1283.56	QPEYDELITSCSSN	HCV NS5 2808	79	93
	1283.57	LELITSCSSNVSAH	HCV NS5 2813	79	100
	1283.58	PTLWARMILMTHFFS	HCV NS5 2870	79	86
	1283.59	LHGLSAFSLHSYSPG	HCV NS5 2919	79	79
	1283.60	AFSLHSYSPGEINRV	HCV NS5 2924	79	79

Table XXXII. Candidate HCV-derived HTL epitopes

Selection criteria	Peptide	Sequence	Source	Conservancy	
				Total	Core
B. High algorithm conserved core	1283.15	VVLLFLILLADARVCS	HCV NS1/2 724	29	100
	1283.16	SKGWRLLAPITAYAQ	HCV NS3 1025	29	79
	1283.19	PQTFQVAHLHAPTGS	HCV NS3 1225	43	85
	1283.26	DVVVVATDALMTGYT	HCV NS3 1436	43	79
	1283.29	WESVFTGLTHIDAHF	HCV NS3 1563	43	92
	1283.45	LTSMLTDPSSHIAET	HCV NS5 2176	57	100
	1283.46	ASQLSAPSLKATCTT	HCV NS5 2208	50	79
	1283.47	DADLIEANLWRQEM	HCV NS5 2232	50	85
	1283.50	SYTWTGALITPCAAE	HCV NS5 2456	64	79
	1283.51	TTIMAKNEVFCVQPE	HCV NS5 2589	64	85
	1283.55	GSSYGFQYSPGQVVE	HCV NS5 2641	71	79
C. Collaborator	1283.61	ASCLRKLGVPLRVW	HCV NS5 2939	50	85
	F098.03	AAYAAQGYKVLVLNPSVAAT	HCV NS3 1242-1261	71	100
	F098.04	GYKVLVLNPSVAATLGFAY	HCV NS3 1248-1267	100	
	F098.05	GYKVLVLNPSVAAT	HCV NS3 1248-1261	100	
	F134.01	RFPQDVKFPGGGQIVGGVY	HCV Core 17-35	86	
	F134.02	DVKFPGGGQIVGGVYLLPRR	HCV Core 21-40	86	
	F134.03	GYKVLVLNPSVAATLGFAY	HCV NS3 1253-1272	100	
	F134.04	TLHGPTLLYRLGAVQNEIT	HCV NS4 1622-1641		79
	F134.05	NFISGIQYLAGLSTLPGNPA	HCV NS4 1772-1791	100	
	F134.06	LLFNILGGWVAAQLAAPGAA	HCV NS4 1812-1831		86
	F134.07	GPGEQAVQWMNRLIAFASRG	HCV NS4 1912-1931	86	100
	F134.08	GEGAQVQWMNRLIAFASRGNHV	HCV NS4 1914-1934	100	
	Pape 21	AIPLEVIKGGRIHIFCHSKR	HCV NS3 1379-1398	21	100
	Pape 22	GRHLIFCHSKRKCDLAKL	HCV NS3 1388-1407		100
D. DR3 motif	Pape 29	SVIDCNTCTVTQVDFSLDPT	HCV NS3 1450-1469	86	
	35.0102	GVRVLEDGVNYATGN	HCV 154	86	86
	35.0103	SAMYVGDLCGSVFLV	HCV 273	57	86
	35.0104	GHRMAWDMMMNWSPT	HCV 315	86	86
	35.0105	SDLVLTVRHADVIPV	HCV 1133	79	86
	35.0106	VVVVATDALMTGYTG	HCV 1437	42	86
	35.0107	TVDFSLDPTFTIETT	HCV 1466	79	100
	35.0108	DSSVLCCEYDAGCAW	HCV 1518	71	93
	35.0109	GLPVCQDHLFEWESV	HCV 1552	42	86
	35.0110	GMQLAEQFKQKALGL	HCV 1726	57	86
	35.0111	PTHVVPESDAAARVT	HCV 1936	86	86
	35.0112	OSQLPCPEPDVAVL	HCV 2162	64	86
	35.0113	LTSMLTDPSSHIAET	HCV 2176	57	100
	35.0114	MPPLEGEGDPLSD	HCV 2401	79	100
	35.0115	QPEYDLELITSCSN	HCV 2808	79	93
	1283.25	GRHLIFCHSKKKCDE	HCV NS3 1393-1407		

Table XXXIII. HLA-DR screening panels

Screening Panel	Antigen	Alleles	Representative Assay		Phenotypic Frequencies						
			Allele	Alias	Cauc.	Blk.	Jpn.	Chn.	Hisp.	Avg.	
Primary	DR1	DRB1*0101-03	DRB1*0101	(DR1)	18.5	8.4	10.7	4.5	10.1	10.4	
	DR4	DRB1*0401-12	DRB1*0401	(DR4w4)	23.6	6.1	40.4	21.9	29.8	24.4	
	DR7	DRB1*0701-02	DRB1*0701	(DR7)	26.2	11.1	1.0	15.0	16.6	14.0	
	Panel total				59.6	24.5	49.3	38.7	51.1	44.6	
Secondary	DR2	DRB1*1501-03	DRB1*1501	(DR2w2 B1)	19.9	14.8	30.9	22.0	15.0	20.5	
	DR2	DRB5*0101	DRB5*0101	(DR2w2 B2)	-	-	-	-	-	-	
	DR9	DRB1*09011,09012	DRB1*0901	(DR9)	3.6	4.7	24.5	19.9	6.7	11.9	
	DR13	DRB1*1301-06	DRB1*1302	(DR6w19)	21.7	16.5	14.6	12.2	10.5	15.1	
	Panel total				42.0	33.9	61.0	48.9	30.5	43.2	
Tertiary	DR4	DRB1*0405	DRB1*0405	(DR4w15)	-	-	-	-	-	-	
	DR8	DRB1*0801-5	DRB1*0802	(DR8w2)	5.5	10.9	25.0	10.7	23.3	15.1	
	DR11	DRB1*1101-05	DRB1*1101	(DR3w11)	17.0	18.0	4.9	19.4	18.1	15.5	
	Panel total				22.0	27.8	29.2	29.0	39.0	29.4	
Quaternary	DR3	DRB1*0301-2	DRB1*0301	(DR3w17)	17.7	19.5	0.4	7.3	14.4	11.9	
	DR12	DRB1*1201-02	DRB1*1201	(DR3w12)	2.8	5.5	13.1	17.6	5.7	8.9	
	Panel total				20.2	24.4	13.5	24.2	19.7	20.4	

Table XXXIV. HLA-DR binding capacity of target derived peptides: DR-supermotif and algorithm positive peptides.

Peptide	Sequence	Source	Binding capacity (IC50 nM)												DR alleles bound
			DR1	DR2w/21	DR2w/22	DR4w4	DR4w15	DR3w/11	DRw/19	DR7	DR8w/2	DR9	IAb		
1283 21	AAVAAQGYKVLINR	NS3 1242-1267	45	350	5.2	567	143	5.1	89	288	54	175	9		
1283 20	AGQRYKVLINR	NS3 1251	6.0	650	7.9	224	74	5.9	833	175	375	298	9		
1283 03	AAVAAQGYKVLINR	NS3 1242	2.9	48	483	18	1234	103	11	96	60	240	9		
1283 05	GYKVLINR	NS3 1248-1261	1.4	39	3993	7.8	141	75	3.5	126	21	266	9		
1283 04	GYKVLINR	NS4 1914-1935	3.5	42	8154	9.7	1500	240	4.1	23	80	20	8		
1283 44	GEQAVQVMNRLIAFASR	NS4 1921	66	48	1538	6329	585	45	7.3	227	102	313	147	8	
1283 08	GEQAVQVMNRLIAFASR	NS4 1914	3.2	182	361	345	345	221	158	6818			6		
1283 16	SRGWMLAPITVAQ	NS3 1025	0.36	125	23	24	152	4.8	199	54	199	384	8		
1283 55	GSSYGQYSPQROVE	NS5 2641	11	667	417	745	2000	19	156	862	671	862	571	7	
1283 61	ASCIIRKLGPPRIAR	NS5 2939	5.0	16	217	6930	78	645	2506	862	671	862	-	7	
1283 05	NRSGIYVLAGLITPQNP	NS4 1772	10	606	84	29	29	29	29	862	671	862	441	6	

Shading indicates IC50 > 1 μM

A dash (-) indicates IC50 > 20 μM

Table XXXV. HLA-DR binding capacity of 3 DR3 motifs-containing peptides

Peptide	Sequence	Source	DR3 binding (IC50 nM)
35.0106	VVVVAITDALMTQYTG	HCV 1437	427
35.0107	TVDFSLDPTLETT	HCV 1466	235
1283.25	GRHLFCHSKKKKDE	HCV NS3 1393	ND

Table XXXVIa: HCV-derived CTL epitope candidates

Peptide	Molecule	1st Position	Sequence	Consv.	Selection criteria
1073.05	NS4	1812	LLFNILGGWV	85	A2-supertype
1090.18	NS1/E2	728	FILLADARV	92	A2-supertype
1013.02	NS4	1590	YLVAVQATV	85	A2-supertype
1090.22	NS5	2611	RLIVPPDLGV	79	A2-supertype
1013.1002	CORE	132	DLMGVPELV	79	A2-supertype
24.0073	NS4	1920	WMNRLAFA	100	A2-supertype
24.0075	NS4	1666	VLVGGVLA	85	A2-supertype
1174.08	NS4	1769	HMWNFISGI	92	A2-supertype
1073.06	NS4	1851	ILAGVGAGV	79	A2-supertype
1073.07	CORE	35	YLLPRKQPL	92	A2-supertype
24.0071	NS1/E2	726	LLFLLLADA	100	A2-supertype
1.0119	LORF	1131	YLVTRHADV	85	A2-supertype
1.0952	CORE	51	KTSFRSOPR	92	A3-supertype
1073.11	CORE	43	RLGVRATRK	79	A3-supertype
1.0955	ENVI	290	QLFTFSPPR	79	A3-supertype
1073.13	NS1/E2	632	RMVVGVEHR	100	A3-supertype
1.0123	NS3	1396	LIFCHSKKK	100	A3-supertype
1073.10	NS4	1863	GVAQALVAFK	85	A3-supertype
24.0090	NS4	1864	VAGALVAFK	85	A3-supertype
24.0086	NS3	1262	TLGFCAYMSK	85	A3-supertype
F104.01	NS5	3003	VGIVLLPNR	79	A31
1145.12	CORE	169	LRGCSRF	92	B7-supertype
29.0035	NS3	1378	IPFYGKAI	92	B7-supertype
13.0019	NS5	2922	LSAFSLHSY	79	A1
1069.62	NS3	1128	CTCCSSDLY	79	A1
24.0092	NS4	1765	FWAKHMWNF	85	A24

Table XXXVIIb: HCV-derived HTL epitope candidates

Region	Peptide	Motif ¹	Sequence
HCV NS3 1025-1039	1283.16	DR	SKGWRIILAPTVAAQ
HCV NS3 1242-1267	F98.03	DR	AAYAAQGYKYVILNPSVAAT
HCV NS3 1393-1407	1283.25	DR3	GRHLIECHSKRKDE
HCV NS3 1437-1451	35.0106	DR3	VVVVATDIALMTGYTG
HCV NS3 1466-1480	35.0107	DR3	TVDFSLDPFTIETT
HCV NS4 1772-1790	F134.05	DR	NFISGIQYILAGLSTLPGNPA
HCV NS4 1914-1935	F134.08	DR	GEGAVQWMNRIILAFASRGNIHV
HCV NS5 2641-2655	1283.55	DR	GSSYGFQYSPGQORVE
HCV NS5 2939-2953	1283.61	DR	ASCLRKILGVPPILRYW

1. Peptides identified on the basis of either the DR P1-P6 superset or by use of the DR1-4-7 algorithms are indicated by 'DR'. Peptides identified using the DR3 motif are indicated by 'DR3'.

Table XXXVII. Estimated population coverage by a panel of HCV derived HTL epitopes

Antigen	Alleles	Representative assay	No. of epitopes ²	Population coverage (phenotypic frequency)						
				Cauc.	Blk.	Jpn.	Chn.	Hisp.	Avg.	
DR1	DRB1*0101-03	DR1	6	18.5	8.4	10.7	4.5	10.1	10.4	
DR2	DRB1*1501-03	DR2w2 B1	3	19.9	14.8	30.9	22.0	15.0	20.5	
DR2	DRB5*0101	DR2w2 B2	6	-	-	-	-	-	-	
DR3	DRB1*0301-2	DR3	2	17.7	19.5	0.40	7.3	14.4	11.9	
DR4	DRB1*0401-12	DR4w4	5	23.6	6.1	40.4	21.9	29.8	24.4	
DR4	DRB1*0401-12	DR4w15	3	-	-	-	-	-	-	
DR7	DRB1*0701-02	DR7	5	26.2	11.1	1.0	15.0	16.6	14.0	
DR8	DRB1*0801-5	DR8w2	5	5.5	10.9	25.0	10.7	23.3	15.1	
DR9	DRB1*0901.1,09012	DR9	3	3.6	4.7	24.5	19.9	6.7	11.9	
DR11	DRB1*1101-05	DR5w11	5	17.0	18.0	4.9	19.4	18.1	15.5	
DR13	DRB1*1301-06	DR6w19	2	21.7	16.5	14.6	12.2	10.5	15.1	
Total ¹				98.5	95.1	97.1	91.3	94.3	95.1	

1. Total population coverage has been adjusted to account for the presence of DRX in many ethnic populations. It has been assumed that the range of specificities represented by DRX alleles will mirror those of previously characterized HLA-DR alleles. The proportion of DRX incorporated under each motif is representative of the frequency of the motif in the remainder of the population. Total coverage has not been adjusted to account for unknown gene types.

2. Number of epitopes represents a minimal estimate, considering only the epitopes shown in Table 6. Additional alleles possibly bound by nested epitopes have not been accounted.

WHAT IS CLAIMED IS

1. A peptide composition of less than 250 amino acid residues comprising a peptide epitope useful for inducing an immune response against hepatitis C virus (HCV) said epitope (a) having an amino acid sequence of about 8 to about 13 amino acid residues that have at least 65% identity with a native amino acid sequence of HBV and, (b) binding to at least one HLA class I HLA allele with an IC_{50} of less than about 500 nM.
2. The composition of claim 1, further wherein said peptide has at least 77% identity with a native HCV amino acid sequence.
3. The composition of claim 1, further wherein said peptide has 100% identity with a native HCV amino acid sequence.
4. A pharmaceutical composition comprising a peptide and a pharmaceutical carrier, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A*0201 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif) comprising an IC_{50} of less than about 500 nM for at least one HLA class I molecule.
5. The pharmaceutical composition of claim 4 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide.
6. The pharmaceutical composition of claim 5 wherein the composition comprises the peptide in a form of nucleic acids that encode the epitope and one or more additional peptide(s).
7. The composition of claim 4, wherein the peptide is comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.
8. The pharmaceutical composition of claim 4 wherein the peptide is in a human dose form, and the carrier is in a human unit dose.

9. A peptide composition of claim 1 comprising an analog of a peptide epitope, wherein the peptide epitope is an epitope of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif), said analog comprising a preferred or less preferred amino acid of Table II substituted in for a starting residue, or having a deleterious residue of Table II substituted out of the starting sequence and replaced by a non-deleterious residue.

10. A peptide composition of claim 1 comprising a peptide of Table XXII.

11. A method for inducing a cytotoxic T lymphocyte response, said method comprising steps of:

providing a peptide that comprises an IC_{50} of less than about 500 nM for an HLA class I molecule, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif); and,

administering said peptide to a human.

12. The method of claim 11, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

13. The method of claim 12, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

14. The method of claim 11, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

15. A method for inducing a cytotoxic T lymphocyte response, said method comprising steps of:

providing a peptide that induces a cytotoxic T cell response *in vitro* and/or *in vivo*, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), Table XVIII (A24 motif) or Table XXIII; and,

administering said pharmaceutical composition to a human.

16. The method of claim 15, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

17. The method of claim 16, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

18. The method of claim 15, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

19. The method of claim 15, wherein the providing step comprises a peptide that induces a cytotoxic T cell response when complexed with an HLA class I molecule and is presented to an HLA class I-restricted cytotoxic T cell.

20. A peptide composition of less than 250 amino acid residues comprising a peptide epitope useful for inducing an immune response against hepatitis B virus (HCV) said epitope (a) having an amino acid sequence of about 6 to about 25 amino acid residues that have at least 65% identity with a native amino acid sequence of HCV and, (b) binding to at least one HLA class II HLA allele with an IC_{50} of less than about 1000 nM.

21. The composition of claim 20, further wherein said peptide has at least 77% identity with a native HCV amino acid sequence.

22. The composition of claim 20, further wherein said peptide has 100% identity with a native HCV amino acid sequence.

23. A pharmaceutical composition comprising:
a human dose form of a peptide of Table XIX or Table XX that comprises an IC_{50} of less than about 1,000 nM for at least one HLA DR molecule of an HLA DR supertype; and,
a human dose of a pharmaceutically acceptable carrier.

24. The pharmaceutical composition of claim 23 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide.

25. The pharmaceutical composition of claim 24 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

26. The composition of claim 25, wherein the peptide is comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

27. A peptide composition of claim 20 comprising an analog of a peptide epitope of Table XIX or Table XX, said analog comprising a preferred or less preferred amino acid of Table III substituted in for a starting residue, and/or having a deleterious residue of Table III substituted out of the starting sequence and replaced by a non-deleterious residue.

28. A method for inducing a helper T lymphocyte response, said method comprising steps of:

providing a peptide that comprises an IC_{50} of less than about 1,000 nM for an HLA class II molecule, wherein the peptide is a peptide of Table XIX or Table XX; and,

administering said peptide to a human.

29. The method of claim 28, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

30. The method of claim 29, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

31. The method of claim 28, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

32. A method for inducing a helper T lymphocyte response, said method comprising steps of:

providing a peptide that induces a helper T cell response *in vitro* and/or *in vivo*, wherein the peptide is a peptide of Table XIX or Table XX; and, administering said pharmaceutical composition to a human.

33. The method of claim 32, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

34. The method of claim 33, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

35. The method of claim 32, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

36. The method of claim 32, wherein the providing step comprises a peptide that induces a helper T cell response when complexed with an HLA class II molecule and is presented to an HLA class I-restricted helper T cell.

37. A vaccine for preventing or treating HCV infection that induces a protective or therapeutic immune response, wherein said vaccine comprises:

at least one peptide selected from Table(s) VII-XX or Table XXII; and,
a pharmaceutically acceptable carrier.

38. A kit for a vaccine that induces a protective or therapeutic immune response to HCV, said vaccine comprising:

at least one peptide selected from Table(s) VII-XX or Table XXII;
a pharmaceutically acceptable carrier; and,
instructions for administration to a patient.

39. A method for monitoring or evaluating an immune response to HCV or an epitope thereof in a patient having a known HLA type, the method comprising:

incubating a T lymphocyte sample from the patient with a peptide selected from Table(s) VII-XX or Table XXII, wherein that peptide bears a motif corresponding to at least one HLA allele present in said patient; and,

detecting the presence of a T lymphocyte that recognizes the peptide.

40. The method of claim 39, wherein the peptide is comprised by a tetrameric complex.

41. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and combination of motif-bearing peptides that are immunologically cross-reactive with hepatitis C virus-1 (HCV-1), wherein at least one of the peptides bears a motif of Table Ia, and further wherein the combination of motif-bearing peptides consists of:

a) one or more peptides comprising at least 8 amino acids from an HCV C domain, the HCV C domain consisting of amino acids 1-120 of the HCV polyprotein;

b) one more peptides comprising at least 8 amino acids of a further domain, wherein the further domain is selected from the group consisting of:

an S domain, the S domain consisting of amino acids 120-400 of the HCV polyprotein;

an NS3 domain, the NS3 domain consisting of amino acids 1050 to 1640 of the HCV polyprotein;

an NS4 domain, the NS4 domain consisting of amino acids 1640 to 2000 of the HCV polyprotein; and,

an NS5 domain, the NS5 domain consisting of amino acids 2000 to 3011 of the HCV polyprotein; and,

42. The composition of claim 41, wherein the composition further comprises one or more additional HCV motif-bearing peptide(s) that are one or more distinct HCV peptides comprising at least 8 amino acids of an X domain, the X domain consisting of amino acids 750 to 1050 of the HCV polyprotein.

43. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and combination of motif-bearing peptides that are immunologically cross-reactive with peptides of hepatitis C virus-1 (HCV-1), the peptides from multiple domains of HCV, wherein at least one of the peptides bears a motif of Table Ia, and further wherein the combination of peptides consists essentially of:

a) one or more peptides comprising at least 8 amino acids from a C domain, the C domain consisting of amino acids 1 to 120 of an HCV polyprotein; and,

b) one or more peptides comprising at least 8 amino acids from an S domain, the S domain consisting of amino acids 120-400 of the HCV polyprotein; or,

one or more peptides comprising at least 8 amino acids from an NS3 domain, the NS3 domain consisting of amino acids 1050 to 1640 of the HCV polyprotein; or,

one or more peptides comprising at least 8 amino acids from an NS4 domain, the NS4 domain consisting of amino acids 1640 to 2000 of the HCV polyprotein; or,

one or more peptides comprising at least 8 amino acids from an NS5 domain, the NS5 domain consisting of amino acids 2000 to 3011 of the HCV polyprotein; and,

c) one HCV peptide comprising at least 8 amino acids of an envelope domain, the envelope domain consisting of amino acids 192 to 750 of the HCV polyprotein.

44. The composition of claim 43, wherein the composition further comprises one or more HCV peptides comprising at least 8 amino acids of an X domain, the X domain consisting of amino acids 750 to 1050 of the HCV polyprotein.

45. A pharmaceutical composition comprising:

a) a pharmaceutically acceptable carrier; and,

b) a combination of one or more motif-bearing peptides of at least 8 amino acids derived from one or more hepatitis C virus (HCV) domains, wherein said motif-bearing peptides are immunologically cross-reactive with peptides of HCV-1, with a *proviso* that the combination does not include a peptide of at least 8 amino acids from an HCV C domain, the C domain consisting of amino acids 1 to 120 of an HCV polyprotein, and wherein at least one of the peptides bears a motif of Table Ia, said domains selected from the group consisting of:

an S domain, the S domain consisting of amino acids 120-400 of the HCV polyprotein;

an NS3 domain, the NS3 domain consisting of amino acids 1050 to 1640 of the HCV polyprotein;

an NS4 domain, the NS4 domain consisting of amino acids 1640 to 2000 of the HCV polyprotein.;

an NS5 domain, the NS5 domain consisting of amino acids 2000 to 3011 of the HCV polyprotein; and,

an X domain, the X domain consisting of amino acids 750 to 1050 of the HCV polyprotein.

46. The composition of claim 45 further comprising:

HCV motif-bearing envelope peptide(s) consisting of one or more copies of a single HCV peptide comprising at least 8 amino acids of an envelope domain, the envelope domain consisting of amino acids 192 to 750 of the HCV polyprotein.

47. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and combination of two or more motif-bearing peptides from a single domain of an hepatitis C virus strain, said peptides immunologically cross-reactive with peptides of a hepatitis C virus 1 (HCV) antigen,

wherein at least one of the peptides bears a motif of Table Ia., and the peptides are derived from HCV, and the HCV domain is selected from the group consisting of:

a C domain, the C domain consisting of amino acids 1 to 120 of an HCV polyprotein;

an S domain, the S domain consisting of amino acids 120-400 of the HCV polyprotein;

an NS3 domain, the NS3 domain consisting of amino acids 1050 to 1640 of the HCV polyprotein;

an NS4 domain, the NS4 domain consisting of amino acids 1640 to 2000 of the HCV polyprotein;

an NS5 domain, the NS5 domain consisting of amino acids 2000 to 3011 of the HCV polyprotein;

an X domain, the X domain consisting of amino acids 750 to 1050 of the HCV polyprotein; and,

an envelope domain, from a single HCV strain, the envelope domain consisting of amino acids 192 to 750 of the HCV polyprotein, with a *proviso* that the envelope domain is other than a variable envelope domain.

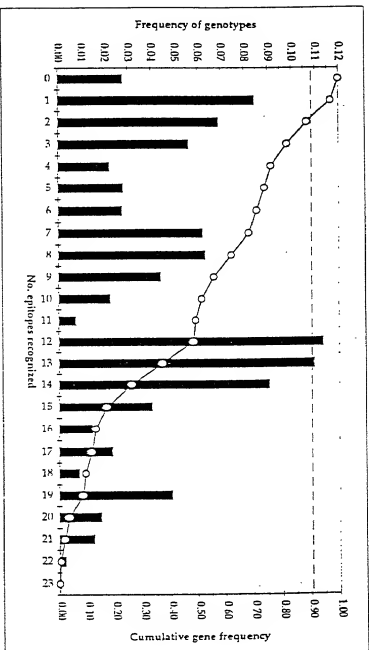
ABSTRACT OF THE DISCLOSURE

This invention uses our knowledge of the mechanisms by which antigen is recognized by T cells to identify and prepare HCV epitopes, and to develop epitope-based vaccines directed towards HCV. More specifically, this application communicates our discovery of pharmaceutical compositions and methods of use in the prevention and treatment of HCV infection.

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Figure 1. Monte Carlo population coverage analysis for
HCV candidate epitopes



Plot of total frequency of genotypes as a function of the number of HCV candidate epitopes bound by HLA-A and B alleles, in an average population. Genotype values were derived by averaging the gene frequencies in Caucasian, North American Black, Japanese, Chinese, and Hispanic populations. Also shown is the cumulative frequency of genotypes.

Using currently available HLA typing data, a residual fraction (about 15%) of the genes, in an average population are unspecified. To arrive at 100% accounting of genes, a fraction of the residual has been added for each in population cluster in proportion to the relative frequency of the cluster within the HLA specified population. One peptide, 24.0066, was not incorporated into the present analysis.

Figure 2

HCV Minigene

CTL Epitopes

Core 43	NS4 1590	NS3 1128	NS5 2611	Core 169	NS1/E2 632	NS4 1765	NS4 1863	Core 132
1073.11	1013.02	1069.62	1090.22	1145.12	1073.13	24.0092	1073.10	1013.10
A3	A2	A1	A2	B/	A3	A24	A3	A2

HTL Epitopes

NS3 1268	NS4 1921	1437	NS5 2041	1466
1283.21	1283.44	35.0106	1283.55	35.0107
DR	DR	DR3	DR	DR3

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **INDUCING CELLULAR IMMUNE RESPONSES TO HEPATITIS C VIRUS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS** the specification of which X is attached hereto or _____ was filed on _____ as Application No. _____ and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
09/189,702	11/10/98	Pending
08/205,713	3/4/94	Pending
08/159,184	11/29/93	Abandoned
08/073,205	6/4/93	Abandoned
08/027,146	3/5/93	Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Signature of Inventor 7 ESTEBAN CELIS Date	Signature of Inventor 8 RALPH T. KUBO Date	Signature of Inventor 9 HOWARD M. GREY Date

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